# THE EFFECT OF SHORT-TERM SOY ISOFLAVONE SUPPLEMENTATION ON mRNA IN LOCALIZED PROSTATE CANCER

BY

Jessica D. Rubin, RD LD

Submitted to the graduate degree program in Dietetics and Nutrition and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science.

Committee Chair:

_____

Jill Hamilton-Reeves, PhD RD LD

Committee Members:

_____

Susan Carlson, PhD

_____

Prabhakar Chalise, PhD

_____

Andrew K. Godwin, PhD

Date defended: 11 July 2013

The Thesis Committee for Jessica D. Rubin
certifies that this is the approved version of the following thesis:

**THE EFFECT OF SHORT-TERM SOY ISOFLAVONE SUPPLEMENTATION ON mRNA IN LOCALIZED PROSTATE CANCER**

Committee Chair:

_____
Jill Hamilton-Reeves, PhD RD LD

Date approved: 11 July 2013

## ABSTRACT

**Background:** Soy isoflavones have been hypothesized to affect gene expression related to prostate cancer development and progression, yet few studies have evaluated the effect of this phytochemical on the transcriptome of prostate cancer cells in a human model.

**Objective:** To determine if short-term soy isoflavone supplementation influences gene expression in men with localized prostate cancer.

**Methods:** Six men diagnosed with clinically localized prostate cancer were asked to take a soy isoflavone supplement for up to 6 weeks prior to radical prostatectomy. Total RNA was isolated and sequenced from tumor and adjacent normal frozen prostate tissue pairs obtained from these participants. Gene expression profiles were determined, differentially expressed genes were identified, and gene expression profiles were mapped to molecular pathways for biological interpretation using Ingenuity Pathway Analysis software (Ingenuity Systems®, www.ingenuity.com). The trimmed mean of the M values method (TMM, where M=log2 fold change) was used to calculate the normalization factor. Quantile-adjusted conditional maximum likelihood (qCML) method was used to estimate common dispersion across all genes. Exact test with a negative binomial distribution was used to calculate expression differences between groups. Multiple test adjustments were carried out using the false discovery rate (FDR) using Benjamini and Hochberg's method. An FDR value of $< 0.05$ was considered statistically significant.

**Results:** All 6 men were included in the final analysis. Soy isoflavones differentially expressed 128 genes in cancerous prostate tissue and 166 genes in normal prostate tissue. Twenty genes were differentially expressed in both tissues, 2 of which were differentially regulated in either tissue. Some pathways suggested a protective effect (human embryonic stem cell pluripotency, complement system, protein citrullination, and methylglyoxal degradation VI pathways), while others suggested harm (intrinsic and extrinsic prothrombing activation and IL-17A pathways). The following pathways were of unknown relevance: acute phase response signaling, UDP-glucuronosyltransferase, and glutamate-dependent acid resistance pathways.

**Conclusion:** Short-term soy isoflavone supplementation prior to curative treatment for localized prostate cancer was found to significantly affect gene expression in tumor and adjacent normal tissues.

**ACKNOWLEDGEMENTS**

Dr. Peter Van Veldhuizen conducted the parent study and provided access to the data. Sarah Spencer assisted with access to the VAMC and provided study documents. Dr. Emma Borrego Diaz Reyes allowed the use of her laboratory to process banked biospecimens. Dr. Safinur Atay isolated the RNA from snap-frozen prostate tissue specimens. Clark Bloomer conducted RNA sequencing and prepared the libraries. Dr. Brooke Fridley and Dr. Prabhakar Chalise conducted statistical analyses. Dr. Byunggil Yoo and Dr. Rama Raghavan converted RNA sequencing data into FastQ files and de-multiplexed into individual sequences. Dr. Yoo also provided access to Ingenuity Pathway Analysis software. Dr. Joshua Griffin provided access to Research Electronic Database Capture. Dr. Jill Hamilton-Reeves, Dr. Susan Carlson, Dr. Andrew Godwin, and Dr. Prabhakar Chalise were members of the thesis committee.

**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURES

## CHATPER I: INTRODUCTION

Prostate cancer remains the most commonly diagnosed non-cutaneous cancer and the second leading cause of cancer deaths in American men (1). In 2013, an estimated 238,590 American men will receive a prostate cancer diagnosis, while approximately 29,720 American men will die from the disease (1). Global incidence data indicates that the highest rates of prostate cancer occur in North America, Australia, and Northern and Western Europe, while the lowest rates occur in Asia and North Africa (2). Although increased screening and health care differences likely contribute to this discrepancy, it is possible that the difference in incidence relates to diet and lifestyle. Population migration studies have shown that prostate cancer incidence increases among Asian men who adopt Western diet and lifestyle patterns after relocating to Western countries (3). Furthermore, population studies have shown that prostate cancer incidence increases among men who adopt Western diet and lifestyle patterns while in their home countries (3). Traditional Asian diet patterns are typically plant-based and include many foods found to contain phytochemicals, which are bioactive compounds thought to play a role in cancer prevention. For example, soybeans, which represent a major protein source in traditional Asian diets, contain bioactive phytochemicals collectively referred to as isoflavones. Replacing the animal proteins emphasized in Western diets with this plant-based protein may explain the difference in prostate cancer rates between Asian and Western countries.

The mechanisms through which soy isoflavones act to affect the development and progression of prostate cancer have been of recent interest. Evidence from cell line

and animal studies suggests that soy isoflavones can affect pathways associated with cell growth, cell cycle progression, and apoptosis (4). Soy isoflavone supplementation in both animal and human models seems to reduce the risk of developing prostate cancer (4); however, the precise mechanism(s) that mitigate this risk have yet to be determined. It is thought that soy isoflavones affect the transcription of certain genes as well as the expression of proteins implicated in prostate cancer development and progression (5,6).

The increasing use of and continued improvements in high-throughput analysis techniques, such as DNA microarrays and massively parallel next-generation sequencing (NGS), have facilitated the rapid discovery of many oncogenes and tumor suppressor genes (7). These technologies have allowed scientists to characterize cancerous tissues, identify differential gene expression between cancerous and normal tissues, investigate the effects of potential therapies, and contribute to a more thorough understanding of the interactions among the genome, epigenome, transcriptome, proteome, and metabolome within the context of prostate cancer. NGS technologies could not only help identify and develop effective prostate cancer treatments, but could also help elucidate the potential chemopreventative effects of dietary components such as soy isoflavones.

**Statement of purpose**

Applying NGS technology to tissue samples inherited from a previous study, the present study will investigate the following question: Does short-term soy isoflavone supplementation influence gene expression in men with localized prostate cancer?

**Objectives**

This exploratory project seeks to characterize RNA sequencing data derived from the NGS analysis of prostate tissue exposed to soy isoflavones or placebo and obtain preliminary data for future studies. The research question will be investigated by addressing the following specific aims:

1. To identify the effect(s) of soy isoflavones on prostate cancer by comparing gene transcripts found in cancerous prostate tissues of men supplemented with soy isoflavones to those of men randomly assigned to a placebo control group.
2. To identify the effect(s) of soy isoflavones on prostate cancer by comparing gene transcripts found in adjacent normal prostate tissues of men supplemented with soy isoflavones to those of men randomly assigned to a placebo control group.
3. To identify the effect(s) of soy isoflavones on prostate cancer by comparing gene transcripts found in cancerous and adjacent normal prostate tissues of men supplemented with soy isoflavones to those of men randomly assigned to a placebo control group.

4. To explore the biological relationships among the most significant of the differentially expressed gene transcripts by conducting pathway analyses using Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com).

The overall hypothesis is that short-term soy isoflavone supplementation will influence the expression of several genes related to prostate cancer development and progression. The goal of the study is to gain insight into the general biological and molecular functions that are altered as a result of soy isoflavone treatment in patients with localized prostate cancer.

**Structure of thesis**

This study seeks to explore the effects of soy isoflavones on the development and progression of prostate cancer by investigating changes in the transcriptome. The review of literature will serve two purposes: 1) to provide background information on soy isoflavones, nutritional genomics, and RNA sequencing technology, and 2) to review the published scientific literature on the effects of dietary bioactive interventions on the transcriptome, while highlighting those that have utilized soy isoflavone interventions. This will be followed by a discussion of the materials and methods used in the present study, and a description of the statistical analyses employed to identify differentially expressed genes. The results of the study will be presented and summarized, followed by a thorough discussion of the significance of these results in the context of the available literature. The thesis will conclude with an evaluation of the study's overall

strengths and weaknesses and will suggest directions for future research. All supplemental information (e.g. tables, illustrations, charts, and graphs) will appear in the appendix.

## CHAPTER II: BACKGROUND AND LITERATURE REVIEW

### Prostate cancer

Although prostate cancer treatments have improved significantly, the disease continues to claim the lives of thousands of men every year (1). When prostate cancer remains localized within the prostate gland, it is surgically removed during a radical prostatectomy procedure. Localized prostate cancer is also treated with radiation therapy. This treatment regimen has reduced the number of prostate cancer deaths by 40% over the past twenty years and resulted in a 5-year survival rate of 100% (8). However, approximately 35% of men continue to experience biochemical recurrence (BCR), i.e. an increase in prostate-specific antigen (PSA) values (the biochemical marker currently used to screen for prostate cancer) following initial treatment, within 5 years (9). Though salvage therapies exist to treat biochemically recurrent disease, they may not effectively cure prostate cancer. In one study, 68% of men with BCR who initially received a prostatectomy and 74% of men with BCR who initially received radiation therapy failed salvage therapy at approximately 3.5 years (10). These men typically develop metastatic disease, characterized by the spread of the initial prostate cancer into distant tissues such as lymph nodes, adrenal glands, lungs, liver, and bone (8,11). Approximately 72% of distant metastatic cancers will result in death within 5 years (8).

Physicians lack the clinical tools that would help them recognize aggressive prostate cancer in order to provide timely and appropriate therapies (7). Currently, physicians evaluate a combination of several clinical characteristics (e.g. preoperative PSA values, Gleason score) to detect prostate cancer and predict the likelihood of BCR

and metastasis. Because this screening methodology lacks specificity and sensitivity, many men remain undiagnosed and under-treated, while others are over-treated. A more thorough understanding of the genetic mechanisms behind prostate cancer development and progression is needed in order to identify biomarkers that can detect aggressive prostate cancer and to apply appropriate treatments (7,12).

The development of, access to, and relative affordability of microarray technologies as well as the accumulation of data from studies using these technologies have allowed for a more comprehensive view of the genes and biochemical pathways expressed in prostate cancer. Comparing gene expression profiles between normal and diseased tissue allows for the identification of candidate biomarkers of prostate cancer (7). For example, the evaluation of data obtained from high-throughput analysis points toward a product of a gene known as EZH2 as a marker of advanced prostate cancer (11). A similar methodology can be applied to prostate cancer tissues that have been exposed to dietary bioactives in order to gain an understanding of diet's effect on genetic expression.

**Nutrigenetics and nutrigenomics**

Though nutrigenetics and nutrigenomics are closely related fields and though they both operate under the overarching hypothesis that health can be improved by tailoring nutrition recommendations to an individual's genetic profile, they are not synonymous: the former refers to the study of how genes affect nutrient uptake and metabolism, while the latter refers to the study of how bioactive food compounds affect

the “omics” (13). The “omics” refer to the study of each stage of genetic replication according to the classical paradigm; therefore, the “omics” include genomics, epigenomics, transcriptomics, proteomics, and metabolomics.

When a cell divides, it replicates its DNA for the new cell. Every cell in the human body carries the same DNA; however, a cell’s function determines the expression of those genes. Genetic expression begins with transcription, a process that involves copying the genetic information encoded within the DNA strand by generating complementary RNA molecules. Three types of RNA are used in the process: messenger RNA, ribosomal RNA, and transfer RNA. Molecules of mRNA determine the order of amino acids; during translation, tRNA molecules use the information encoded in the mRNA to select the correct amino acid for the growing protein chain, while rRNA fuses the amino acids together to form a complete protein product (14).

The number and type of transcripts within a cell is termed the transcriptome, and it can be used to describe the cell. All cells contain the same DNA, but the function of the cell affects the transcriptome. Quantifying the transcripts present in a cell indicates which genes are being expressed in that cell at that point in time (14). By comparing the transcripts of normal and diseased cells, transcriptomic technologies can help identify which genes are being expressed or silenced by cancerous processes. Similarly, by comparing the transcripts of cells exposed to dietary bioactives to those of unexposed cells, transcriptomic technologies may identify which protective genes are being up-regulated by the chemical or which cancer-promoting genes are being down-regulated by the chemical.

**Transcriptomics**

Over the past several years, technology for evaluating the transcripts of cells has evolved, improved, and become more financially feasible. Transcriptomic studies characterize and quantify the RNA content of a given cell or tissue sample, use the data to deduce gene expression, and determine the biological significance of differentially expressed genes by interpreting them within the context of established molecular pathways (15). Real-time quantitative PCR (qPCR), microarrays, and massively parallel next-generation RNA sequencing, or RNA-seq, are the three major techniques for examining the transcriptome (16). The qPCR method is most appropriate for looking at a small number of transcripts (16). Microarrays and RNA sequencing can identify transcripts expressed across the entire genome (16). Microarrays can only detect sequences already specified on the array, while RNA sequencing technologies can detect all mRNA sequences as well as microRNAs, long non-coding RNAs, and strand-specificity (16). Because of this, RNA sequencing technologies are best suited for exploratory studies such as the present study. RNA sequencing applications include Illumina's TruSeq Stranded mRNA Sample Preparation Kit, Life Technologies' SOLiD® Total RNA Seq Kit, and Agilent's SureSelect Strand Specific RNA Library Preparation Kit (16).

Essentially, RNA-seq consists of five distinct phases: 1) isolating the RNA from the sample, 2) preparing a library of cDNA molecules, 3) sequencing by synthesis, 4) aligning the raw reads to a reference transcriptome or genome, and 5) determining statistical significance for differential gene expression. The identification of differentially

expressed genes helps determine the function of genes as the cells respond to different treatments or conditions.

RNA-Seq produces large, complex data sets. The acquisition, management, storage, retrieval, and analysis of these data sets represent significant challenges when working with RNA sequencing data (13). The high cost of RNA sequencing limits the number of samples that can be analyzed; therefore, comparing different doses and different time points can rarely be examined and only general effects can be identified (17). Transcriptomic studies also vary considerably in terms of experimental design, microarray technology, statistical methodology, patient characteristics, and dietary bioactive (7,15), making it somewhat difficult to draw meaningful conclusions from the available literature. The microarray technology itself further complicates matters as the reliability of data can be compromised by the source (e.g. cell lines or human tissue) and quality of mRNA (17,18). Although "omics" research continues to contribute novel data, results must be placed within the context of the each subject's nutrition status, lifestyle habits, clinical profile, physiological conditions, demographics, and environmental factors (13).

**Soybeans**

Soybeans, a plant native to Southeast Asia, are legumes that represent a high-quality, complete protein source (19). Soybeans may be steamed, roasted, or processed into different soy-based products, ranging from more traditional soy foods (e.g. tofu, miso, tempeh, and soy milk) to "second generation" soy-based infant formulas,

meatless soy burgers, soy protein isolate, soy flour, and various food additives (20). Even though traditional soy products are a staple of Asian cuisine, North American soy food sales have increased $1 billion to $5.2 billion over the past 15 years (21). The United Soybean Board reported that 37% of Americans consumed soy products at least once a month in 2011 (21). On average, however, Americans and Europeans consume less than 1 gram of soy protein per day. Conversely, Asian cultures consume much more soy protein than Americans and Europeans. The Japanese consume 8.7 g daily; Koreans, 6.2-9.6 g; Indonesians, 7.4 g; and the Chinese, 3.4 g (20,22).

Soybeans contain several bioactive compounds, including isoflavones. Isoflavones belong to a class of phytochemicals called phytoestrogens, a group of compounds with chemical structures resembling that of estrogen (20). Though isoflavones occur in other plants, soybeans represent the most commonly consumed source of dietary isoflavones in the American diet. Soybeans contain three major isoflavones: genistein, daidzein, and glycitein. Within unprocessed soybeans, these isoflavones are bound to sugar molecules, present as the biologically inactive compounds genistin, daidzin, and glycitin (20). During digestion, isoflavones undergo separation from these sugars and become the biologically active isoflavones, usually referred to as aglycone isoflavones (20).

The isoflavone content in soybeans and soy-based products varies according to their growing environment, the amount and type of food processing, and individual variation in human digestion, among other factors. For example, 3.3 oz steamed soybeans (i.e. edamame) contains ~50 mg isoflavones, while the same amount of

cooked soybeans contains ~18 mg isoflavones (23). Soy protein concentrate produced by alcohol extraction has only ~11.5 mg isoflavones per 3.3 oz, while soy protein concentrate produced by aqueous extraction has ~95 mg isoflavones for the same amount (23). Additionally, daidzein can be further metabolized into equol, a metabolite that may elicit a biological effect in the intestines of approximately 30% of Americans (24). Because soy protein is more frequently consumed among Asian populations than Western populations, it stands to reason that isoflavone intake is also higher in Asian populations than Western populations. On average, the Japanese consume approximately 27 mg isoflavones, or one serving of soy food, daily (25–27). In contrast, those consuming a typical Western diet consume an average of 2-3 mg isoflavones per day (28).

**Potential mechanisms of action**

Many *in vitro* and *in vivo* studies have been conducted to determine the potential mechanisms of action through which soy isoflavones exert their hypothesized chemopreventative effects; however, data remain inconclusive as study designs vary widely in terms of isoflavone dose, form of administration, or timing and duration of exposure (17). Unfortunately, many *in vitro* studies tend to supply isoflavones at concentrations that exceed those that could be achieved in human plasma or tissues by tolerable oral intake (29). Steiner et al. (17) report that isoflavone concentrations as high as 1-2.5 nmol/g can be achieved in prostate tissue after short-term supplementation. This complicates the interpretation of any anti-carcinogenic effect

produced by soy isoflavones in such studies, as any preventative effect seen would be impossible to obtain through the diet (17).

Despite this variability, isoflavones have been shown to modulate steroid synthesis, transport, and metabolism; inhibit protein tyrosine kinases; modify nuclear receptor binding, hormone receptor binding, and other cell signaling pathways; induce apoptosis, anti-oxidant effects, and cell cycle arrest; and suppress metastasis and angiogenesis (17,30). High-throughput data analysis techniques are being used to investigate these effects more closely by attempting to identify how soy isoflavones regulate the expression of genes related to these observed effects involved in prostate cancer prevention, development, and progression. Published studies that investigate the effects of nutritional interventions on the transcriptome vary considerably in terms of dietary bioactive, matrix, and dose; biological specimen, medical condition of interest, population, and sample size; intervention duration; microarray platform (i.e. RNA methods); as well as statistical techniques and software tools used to evaluate biological significance.

**Effects of bioactive dietary components on the transcriptome**

A transcriptomic approach has been used to investigate the influence of various bioactives on genes related to various medical conditions. In addition to prostate cancer, the medical conditions represented in this review include obesity, weight loss, metabolic syndrome, hyperlipidemia, cardiovascular disease, osteoporosis, zinc deficiency, and colon cancer. The transcripts of healthy individuals were also evaluated.

With the exception of soy isoflavones in prostate cancer, this review revealed little consistency among the dietary bioactives used. Some *in vivo* studies took advantage of the micronutrients and phytochemicals already present in or derived from different foods, including the anthocyanins and flavonols in dried and pureed bilberries (31), the zinc content of various foods (32), the sulforaphane content in broccoli (33), and the isoflavone content of tofu (34). Other *in vivo* studies artificially supplemented diets or provided dietary supplements to subjects. Rodents were typically supplemented per kg body weight or per kg diet (35–37), while humans received capsules containing bioactive substances (30,38–41) or oral dietary supplements (32). *In vitro* studies exposed established cell lines or cells derived from harvested tissues to purified concentrations of micronutrients or phytochemicals, including sulforaphane (33,42), curcumin (43), kuguacin-J (44), genistein (41,45–49), daidzein (30), and equol (30).

The studies that implemented soy isoflavone interventions in prostate cancer (n = 6) used purified isoflavone solutions (30,41,46–49) or isoflavone-containing capsules (30,41). The isoflavone solutions used for *in vitro* studies ranged in concentration from 0.0075 µM to 100 µM per liter (30,41,46–49). In one of the human studies, participants consumed a pill containing 27.2 mg mixed isoflavone aglycones three times daily, or approximately 82 mg mixed isoflavones daily (30). In the other human study, participants consumed a capsule containing 150 mg genistein only (40).

Though the attempt was made to focus on human interventions, this literature review also included *in vitro* studies and rodent models when needed. Studies of various bioactives in various medical conditions (n = 5) focused exclusively on human

interventions. Sample sizes were generally limited, ranging from 3 (31,38,42) to 16 participants (39). The source of the biospecimen obtained from these participants also varied, including tumor and adjacent normal colon tissue (42), cheek cells (32), and blood (31,32,38,39). Notably, the majority of these studies used biospecimens that were relatively non-invasive and easy to obtain from human participants.

Studies of various bioactives on the prostate cancer transcriptome used LNCaP cells (33,43), PC-3 cells (44), C4-3B cells (43), and cells derived from the tumor tissue of Copenhagen rats with MatLyLu Dunning-induced prostate cancer (35). Studies that investigated the effect of soy isoflavones on the transcriptome of various tissue types employed both rodent (34,36,37,45,50) and human models (40), including female Zucker rats, male and female Sprague-Dawley rats, and healthy post-menopausal women. Prostate cancer cell lines, specifically LNCaP cells (30,47,49), PC-3 cells (30,41,46,48,49), PC-3-M cells (41), DU-145 cells (48), 1532-NPTX (41), 1542-NPTX (41), 1532-CPTX (41), 1542-CPTX (41), and RWPE-1 cells (49), were used in studies of soy isoflavones on the prostate cancer transcriptome. The two studies with human participants (30,41) used prostatectomy tissue samples obtained from male prostate cancer patients who consumed a soy isoflavone supplement prior to surgery.

Although the duration of the nutritional interventions varied considerably, the majority were relatively short. The majority of rodent studies provided dietary intervention for 2 weeks (34,37,51), while others continued for 4 weeks (41), 6 weeks (35), 12 weeks (36,45), or 40 weeks (50). Prostate cancer cell lines were exposed to various bioactive dietary supplements for 2 to 96 hours, and at regular time points

within this range (30,33,41–44,46–49). Human dietary interventions ranged from 2 weeks (30,38,41) to 11 weeks (40). The *in vitro* studies that implemented soy isoflavone interventions in prostate cancer lasted between 6 (46) and 96 hours (47), while both human studies lasted between 2 and 6 weeks (30,41). All reviewed studies reported statistically significant results, suggesting that dietary supplementation can effect a change in genetic expression in a relatively short time period.

None of the studies reviewed used massively parallel next-generation sequencing techniques to evaluate the effects of dietary bioactives on the transcriptome. Many used qPCR to evaluate the transcriptome, some of which correlated microarray data with the results of the more established qPCR technique (30–32,34,36,37,39,41,44–47,49,51). Many others used microarrays (33,35,38,40,42,43,46,48,50,51). The most common microarray used was Affymetrix GeneChip® Gene Expression (35,38,42,43,48,50,51). Because the present study uses next-gen technology, it will be able to more thoroughly evaluate the transcriptomic response of prostate cancer to soy isoflavones.

**Summary**

A review of the relevant published literature indicates that micronutrient and other dietary bioactive compounds can induce a change in the transcriptome of cells derived from various tissues in as little as 2 weeks. These studies illustrate how evaluating the transcriptome of various cells can be used to investigate the effects of micronutrients and other dietary bioactives. Importantly, significant results were

discovered despite the fact that many of these studies used physiological doses of their respective supplements. Human transcriptomic studies are few, especially when focusing specifically on soy isoflavones and prostate cancer, and most of these had a very limited sample size.

The present study seeks to explore the effect of soy isoflavones on gene expression in prostate cancer using the insights provided by RNA sequencing applications. Gene expression profiles will be obtained by isolating and sequencing the RNA of fresh frozen prostate tissue collected from 6 participants who underwent radical prostatectomy for the treatment of localized prostate cancer. These participants received either a soy isoflavone supplement that provided 51 mg total aglycone equivalents per day or a matching placebo capsule for up to 6 weeks before the operation. The transcripts of normal and tumor tissue from each participant will be quantified and subsequently mapped to known genes. Differentially expressed genes will be identified. The biological relationships among these genes will be evaluated and interpreted using Ingenuity Pathway Analysis software (Ingenuity Systems ®, www.ingenuity.com). The list of highly expressed genes will be used to further explore the effect of soy isoflavones on genetic expression in prostate cancer.

## CHAPTER III: MATERIALS AND METHODS

### Study overview

Under a protocol approved by the Institutional Review Boards at the University of Kansas (KUH) and the Kansas City Veterans Affairs Medical Center (VAMC), 86 men diagnosed with prostate cancer who were scheduled to undergo prostatectomy were enrolled in the study after providing informed consent. Eligibility criteria included: histologically confirmed prostate adenocarcinoma, clinically localized disease (stage T1 or T2), and medical clearance for surgery. Exclusion criteria included: concurrent chemotherapy, radiation, or hormone therapy, consumption of soy foods within 90 days prior to enrollment or during the study, initiation of any new vitamin, mineral, or herbal supplement during the study, and a known history of soy allergy or intolerance. The study was a double-blinded, randomized, placebo-controlled trial in which participants were asked to take study capsules for up to 6 weeks before operation. Participants were randomized to receive either a soy isoflavone supplement (n = 42) or a matching placebo (n = 44). Between April 2006 and May 2009, each participant provided biological specimens for future analysis, including urine samples and snap frozen prostate tissue (both normal and tumor samples), which were subsequently banked at the VAMC.

The current study focused on a subsample (n = 6) of the original 86 participants, and analyzed and sequenced total RNA isolated from tumor and adjacent normal frozen prostate tissue obtained from these participants **(Figure 1)**. From the RNA sequencing data, gene expression profiles were determined, differentially expressed genes were

identified, and gene expression profiles were mapped to molecular pathways for biological interpretation.

## Materials

### *Soy isoflavone and placebo capsules*

The treatment group received a commercially available, soy isoflavone capsule (Flav-ein, 3 B'S Ltd., Lenexa, KS) derived from soy germ that provided 51 mg total isoflavone aglycone equivalents per day, with an average distribution of 55% daidzein, 30% glycitein, and 15% genistein as analyzed by an independent laboratory (NP Analytical Laboratories, St. Louis, MO). The placebo pills were manufactured to appear identical to the isoflavone capsules and provided <0.06 mg isoflavones per day (NP Analytical Laboratories, St. Louis, MO).

## Methods

### *Urine sample collection and analysis for compliance*

Urine samples were collected at baseline, 2 weeks, and immediately prior to prostatectomy. Samples were stored at -80˚C without preservative. Urine samples collected between 2 and 4 weeks from baseline were selected for isoflavone analysis. Frozen urine samples were thawed overnight, aliquoted into 2-mL tubes, packed in dry ice, and shipped. The urinary concentration of 3 isoflavones and 3 isoflavone metabolites (genistein, daidzein, glycitein, equol, dihydrodaidzein, and O-desmethylangolensin) were measured at the University of Minnesota using a high

pressure liquid chromatography-tandem mass spectrometry (LC-MS/MS) method based on a previously used method (52). Urinary creatinine was used to normalize the urinary isoflavone data because 24-hour urinary excretion volume was unknown. The Kansas City VAMC determined urinary creatinine excretion using a kinetic alkaline picrate method (Abbott 8200, Abbott Laboratories, Abbott Park, IL).

Because the isoflavone supplement provided proportionally more daidzein than isoflavone supplements derived from whole soybeans, urinary daidzein concentrations were used to determine intervention compliance. Those consuming placebo capsules were considered non-compliant if their adjusted urinary daidzein concentration exceeded 10 nM/mL. This level was chosen based on a natural cutoff-point as well as normal concentrations found in the United States. Those consuming soy supplement capsules that had low urinary daidzein levels were considered compliant as these reduced levels were likely related to non per os (NPO), or nothing by mouth, status prior to surgery. Equol producer status was classified according to a published method (53).

***Prostate tissue collection and analysis***

Prostate tissue samples were obtained from patients at the Kansas City VAMC. The urological surgeon obtained core samples of both tumor and adjacent normal prostate tissue during the prostatectomy procedure. The samples were immediately snap-frozen, stored in liquid nitrogen, and transferred to KUMC by the KUMC biospecimen repository team, where Dr. Ossama Tawfik (University of Kansas Medical Center, Kansas City, KS) reviewed and graded each core specimen in order to ensure the

presence of tumor or normal tissue. Samples were stored at -80˚C and were transferred to liquid nitrogen prior to RNA isolation and sequencing.

***RNA sequencing***

Total RNA was isolated from 6 normal/tumor pairs of flash-frozen prostate tissue samples from 6 participants at KUMC using TRIzol® reagent with Phase Lock GelHeavy (54). These 6 participants were chosen because they had normal/tumor pairs in inventory **(Table 1)**. Depending on the amount of biopsy available, between 20-50 mg of tissue were used for total RNA isolation. The tissue was homogenized with 0.8-1 ml TRIzol® reagent (50-100 mg tissue). The cell lysate was added to pre-spun Phase Lock GelHeavy tubes and incubated for 5 minutes at 25˚C. Chloroform was added to the tubes at 0.2 ml chloroform per 1 ml TRIzol® reagent and tubes were centrifuged at no more than 12,000 x g for 10 minutes at 4˚C. The aqueous phase containing the RNA was transferred to a fresh tube. RNA was precipitated by adding 0.5 ml isopropyl alcohol per 1 ml TRIzol® reagent used initially. Samples were mixed by repeated inversion, incubated at 25˚C for 10 minutes, and centrifuged for 10 minutes at no more than 12,000 x g at 4˚C. The supernatant was decanted, and 1 ml 75% ethanol per 1 ml TRIzol® reagent was added to wash the RNA pellet. The samples were mixed to dislodge the pellet and were centrifuged at no more than 7,500 x g for 5 minutes at 4˚C. The supernatant was again decanted. The RNA pellets were air-dried for 5-10 minutes to remove any residual ethanol, dissolved in molecular biology grade water, and incubated at 55˚C for 10 minutes to facilitate dissolution.

Total RNA extracted from the samples was multiplexed (3 samples per lane) and sequenced at a 50 bp paired-end resolution using Illumina® HiSeq® 2500 Sequencing System (University of Kansas Medical Center Genomics Core, Kansas City, KS). Total RNA (0.5 µg) was used to initiate the RNA sequencing library preparation protocol (55). The mRNA fraction was enriched, sized, reverse-transcribed into cDNA and ligated with the appropriate indexed adaptors using Illumina® TruSeq™ RNA Sample Preparation Kit v2 (Illumina RS-122-2001/2002). Following Agilent 2100 Bioanalyzer QC, the libraries were adjusted to a 2 nM concentration and pooled for multiplexed sequencing. Libraries were denatured and diluted to 13 pM followed by clonal clustering onto the sequencing flow cell using the TruSeq™ Paired-End (PE) Cluster Kit v3-cBOT-HS (Illumina PE401-3001). The clonal clustering procedure was automated using the Illumina® cBOT Cluster Station. The clustered flow cell was sequenced on the Illumina® HiSeq® 2500 Sequencing System using the TruSeq™ SBS Kit v3-HS (Illumina FC401-3002).

Following collection, RNA sequencing data were converted from .bcl file format to FastQ files and de-multiplexed into individual sequences (University of Kansas Medical Center Bioinformatics Core, Kansas City, KS). A secure FTP site was created and the data were made available for download. Short reads were quality assessed using FastQC (Version 0.10.1, Babraham Bioinformatics, Cambridge, United Kingdom) (56), a quality control tool for raw high throughput sequence data. Short reads were mapped to the GRCh37 assembly (UCSC name: hg19) using TopHat (Version 2.0.8b, Center for Bioinformatics and Computational Biology, College Park, MD) (57), a fast splice junction mapper for RNA-Seq reads that aligns RNA-Seq reads to mammalian-sized genomes and

analyzes mapping results to identify splice junctions between exons. Read alignments were quality assessed using sam-stats (October 2012) (58), a tool used for computing statistics from SAM or BAM files, and RNA-SeQC (Version 1.1.7) (59), a program that computes a series of quality control metrics for RNA-seq data. Read counting was performed using htseq-count (Version 0.5.4p2) (60). Gene annotation files were downloaded from Ensembl 70 (Feb 2013 release) (61).

***Statistical Analysis***

Separate analyses were carried out for tumor data and normal data. The results of the two analyses were further summarized using the set of differentially expressed genes commonly expressed in both normal and tumor tissues. Any gene that had zero mapped reads for all six samples were removed. Because RNA sequencing data is count data, discrete probability distribution based models were used to analyze the data. Because one of the characteristics of RNA sequencing data is that observed variation is greater than the mean, negative binomial distribution was used.

Normalization was performed to adjust for varying sequencing depths and potentially other technical effects across replicates. The trimmed mean of the M values method (TMM, where M=log2 fold change) was used to calculate the normalization factor. Quantile-adjusted conditional maximum likelihood (qCML) method was used for estimating common dispersion across all genes. Then, exact test with a negative binomial distribution was used to calculate expression differences between the soy and placebo groups. Multiple test adjustments were carried out using the false discovery

rate (FDR) using Benjamini and Hochberg's method. The analyses were carried out using R version 3.0. Bioconductor Package edgeR (R Core Team, Vienna, Austria) was used to complete the analyses (62). An FDR value of < 0.05 was considered statistically significant. Statistical analysis was conducted by Dr. Prabhakar Chalise (University of Kansas Medical Center Department of Biostatistics, Kansas City, KS).

***Bioinformatics***

Pathway analysis used Ingenuity Pathway Analysis (IPA) (Ingenuity® Systems, www.ingenuity.com) for all differentially expressed genes (FDR < 0.05). To determine if differentially expressed genes were relevant to the prostate cancer disease process, each set of differentially expressed genes (genes in tumor and normal tissue and the common genes shared between normal and tumor samples) were uploaded into IPA. The canonical pathways and molecules for each set of differentially expressed genes were downloaded from IPA. The top canonical pathways and molecules for comparison analyses were also downloaded from IPA.

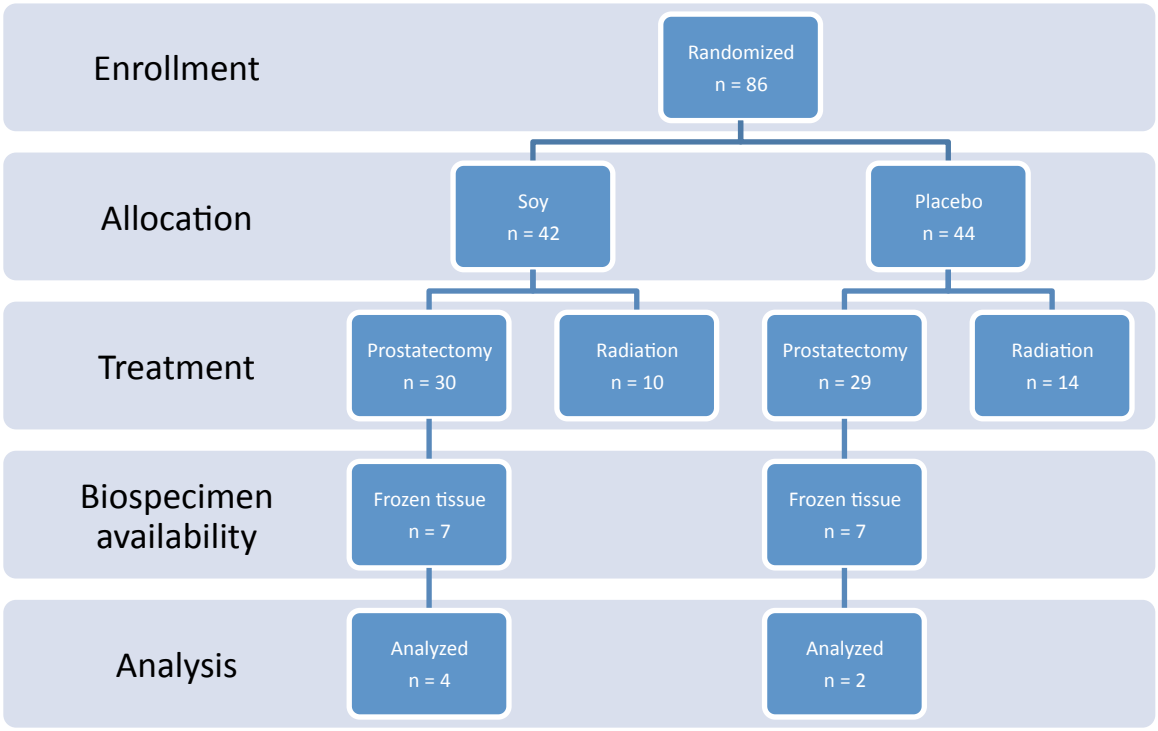
***Electronic medical record abstraction***

Clinical outcome data were abstracted from electronic medical records (EMR) at the Kansas City VAMC. Patient charts were reviewed for demographic information, treatment modalities and complications, clinical and pathological staging, Gleason score, comorbidities, PSA, biochemical recurrence, follow-up status, and survival status.

**Data collection**

Baseline demographic information, clinical outcome data, and the results of subsequent biospecimen analyses have already been collected and reported on this sample (63). All data received from urinalysis, tissue analysis, and EMR abstraction were collected and maintained using Research Electronic Data Capture (REDCap version 5.3.4) hosted on a HIPAA-certified server at the University of Kansas. RNA sequencing results were de-identified, exported into Microsoft© Excel spreadsheets, and stored on a secure drive on the KUMC campus. IPA results were de-identified, exported into Microsoft© Excel spreadsheets and jpeg files, and stored on secure computers.

**Figure 1. Participant flow diagram.** The figure shows the current study's participants relationship to the parent study's participants.



## CHAPTER IV: RESULTS

### Subject characteristics

Of the 86 men originally randomized in the parent study, 42 were allocated to receive soy isoflavone supplementation and 44 were allocated to receive the matching placebo capsule. Of the 42 men supplemented with soy isoflavones, 30 men (71%) underwent radical prostatectomy; 29 men (66%) underwent radical prostatectomy in the placebo group. Two men, one from each treatment group, chose active surveillance in lieu of surgery or radiation therapy. Tissue was collected from 14 of the 59 men who chose surgery. In some cases, the tumor was too small or the KUMC biospecimen repository team missed the tumor in collection. Out of the 14 tissue samples, only 6 had adequate normal/tumor pairs **(Table 1, Table 2)**.

Therefore, only 6 prostate tissue samples were used for analysis: 4 from men who received isoflavone supplementation, and 2 from men who received placebo. Baseline characteristics of the subset (n = 6) appear in **Table 3**.

### Urinary isoflavone levels

Urine samples from all 6 participants were analyzed for urinary isoflavone and isoflavone metabolite concentrations to indicate compliance with the supplementation protocol **(Table 4)**. All 6 participants were compliant with the protocol. Data on equol production were known for all 6 participants included in the analysis. Out of the 4 participants taking soy supplements, only Patient No. 2 was an equol producer according to the equol-to-daidzein ratio >0.018 definition (53).

**Differential gene expression**

A total of 62,069 genes were obtained from the RNA sequencing data. In the 6 tumor tissue samples, 20,209 genes had zero mapped reads for all samples. These were removed from analysis, leaving 41,860 genes for analysis. Of these, 128 genes were found to be differentially expressed between soy and placebo in the tumor tissue samples. Out of the 128 differentially expressed genes, 93 were down-regulated and 35 were up-regulated in the soy group when compared to the placebo group **(Table 5, Table 6)**. Of these 128 differentially expressed genes, 108 were expressed only in tumor tissue **(Table 7, Table 8)**.

In the 6 normal tissue samples, 16,471 genes had zero mapped reads for all samples, leaving 45,598 genes for the analysis. Of these, 186 were found to be differentially expressed between soy and placebo groups in the normal tissue samples. Out of the 186 differentially expressed genes, 147 were down-regulated and 39 were up-regulated in the soy group when compared to the placebo group **(Table 9, Table 10)**. Of these 186 differentially expressed genes, 166 were expressed only in normal tissue **(Table 11, Table 12)**.

A comparison of the 128 differentially expressed genes in tumor tissue samples to the 186 differentially expressed genes in normal tissue samples revealed 20 genes in common between the two groups **(Table 13)**. Of the 20 genes expressed in both tissues, 11 were down-regulated and 9 were up-regulated in normal tissue. Similarly, 11 were down-regulated and 9 were up-regulated in tumor tissue. The regulation direction of

only 2 of the 20 genes differed between normal and tumor tissue **(Table 14, Table 15, Table 16)**.

**Pathway analyses**

***Tumor tissue***

The top five canonical pathways enriched for the 128 differentially expressed genes in tumor tissue were: extrinsic prothrombin activation pathway (2 of the 128 genes were in the list of 16 genes within the pathway; $p = 2.56x10^{-3}$ for enrichment of pathway), human embryonic stem cell pluripotency (4 of the 128 genes were in the list of the 149 genes within the pathway; $p = 3.6x10^{-3}$ for enrichment of pathway), intrinsic prothrombin activation pathway (2 of the 128 genes were in the list of 32 genes within the pathway; $p = 7.78x10^{-3}$ for enrichment of pathway), acute phase response signaling (4 of the 128 genes were in the list of 173 genes within the pathway; $p = 8.39x10^{-3}$ for enrichment of pathway), and the complement system (2 of the 128 genes were in the list of 33 genes within the pathway; $p = 1.01x10^{-2}$ for enrichment of pathway) **(Table 17, Figure 2)**.

The 2 differentially expressed genes up-regulated by soy isoflavones within extrinsic prothrombin activation and intrinsic prothrombin activation were FGA ($p = 4.28x10^{-5}$) and FGB ($p = 2.78x10^{-7}$) **(Figure 3)**. The 4 differentially expressed genes within human embryonic stem cell pluripotency were BMP5 (down-regulated; $p = 6.66x10^{-16}$), NANOG (down-regulated; $p = 1.40x10^{-4}$), WNT2 (down-regulated; $p = 9.60x10^{-6}$), and ZIC3 (up-regulated; $p = 1.29x10^{-4}$) **(Figure 3)**. The 4 differentially expressed genes within

acute phase signaling were FGA (up-regulated; $p = 4.28x10^{-5}$), FGB (up-regulated; $p = 2.78x10^{-7}$), LBP (down-regulated; $p = 1.07x10^{-4}$), and RBP4 (down-regulated; $p = 1.46x10^{-6}$) **(Figure 3)**. The 2 differentially expressed genes within the complement system were CFD (down-regulated; p $9.99x10^{-5}$) and CR2 (down-regulated; $2.54x10^{-8}$) **(Figure 3)**.

***Normal tissue***

The top five canonical pathways enriched for the 186 differentially expressed genes in normal tissue were: the role of IL-17A in psoriasis (2 of the 186 genes were in the list of 13 genes within the pathway; $p = 1.10x10^{-3}$ for enrichment of pathway), the methylglyoxal degradation VI pathway (1 of the 186 genes were in the list of 1 gene within the pathway; $p = 3.84x10^{-3}$ for enrichment of pathway), glutamate dependent acid resistance (1 of the 186 genes were in the list of 2 genes within the pathway; $p = 7.67x10^{-3}$ for enrichment of pathway), GABA receptor signaling (2 of the 186 genes were in the list of 46 genes within the pathway; $p = 1.35x10^{-2}$ for enrichment of pathway), and protein citrullination (1 of the 186 genes were in the list of 5 genes within the pathway; $p = 1.91x10^{-2}$ for enrichment of pathway) **(Table 18, Figure 4)**.

The 2 differentially expressed genes up-regulated by soy isoflavones within the role of IL-17A in psoriasis were CXCL5 ($p = 3.54x10^{-6}$) and S100A7 ($p = 1.55x10^{-5}$) **(Figure 5)**. The differentially expressed gene down-regulated by soy isoflavones within the methylglyoxal degradation VI pathway was LDHD ($p = 6.06x10^{-5}$) **(Figure 5)**. The differentially expressed gene down-regulated by soy isoflavones within glutamate dependent acid resistance was GAD2 ($p = 2.92x10^{-14}$) **(Figure 5)**. The 2 differentially

expressed genes down-regulated by soy isoflavones within GABA receptor signaling were GABRA4 ($p = 7.79x10^{-5}$) and GAD2 ($p = 2.92x10^{-14}$) **(Figure 5)**. The differentially expressed gene down-regulated by soy isoflavones within protein citrullination was PADI3 ($p = 1.04x10^{-4}$) **(Figure 5)**.

***Overlapping pathways***

The top five canonical pathways enriched for the 20 differentially expressed genes in common between normal and tumor tissue were: extrinsic prothrombin activation pathway (1 of the 20 genes were in the list of 16 genes within the pathway; $p = 7.28x10^{-3}$ for enrichment of pathway), thyroid hormone metabolism II (1 of the 20 genes were in the list of 26 genes within the pathway; $p = 1.18x10^{-2}$ for enrichment of pathway), intrinsic prothrombin activation pathway (1 of the 20 genes were in the list of 32 genes within the pathway; $p = 1.27x10^{-2}$ for enrichment of pathway), the coagulation system (1 of the 20 genes were in the list of 35 genes within the pathway; $p = 1.59x10^{-2}$ for enrichment of pathway), and nicotine degradation III (1 of the 20 genes were in the list of 45 genes within the pathway; $p = 2.03x10^{-2}$ for enrichment of pathway) **(Table 19, Figure 6)**.

The 2 differentially expressed genes up-regulated by soy isoflavones were FGB and UGT2B4 **(Table 20)**. The differentially expressed gene up-regulated by soy isoflavones within the extrinsic prothrombin activation pathway was FGB ($p = 2.78x10^{-7}$ in tumor tissue; $p = 1.11x10^{-6}$ in normal tissue). The differentially expressed gene up-regulated by soy isoflavones within the thyroid hormone metabolism II pathway was

UGT2B4 ($p = 4.06x10^{-5}$ in tumor tissue; $p = 1.68x10^{-6}$ in normal tissue). The differentially expressed gene up-regulated by soy isoflavones in the intrinsic prothrombin activation pathway was FGB ($p = 2.78x10^{-7}$ in tumor tissue; $p = 1.11x10^{-6}$ in normal tissue). The differentially expressed gene up-regulated by soy isoflavones within the coagulation system was FGB ($p = 2.78x10^{-7}$ in tumor tissue; $p = 1.11x10^{-6}$ in normal tissue). The differentially expressed gene up-regulated by soy isoflavones within the nicotine degradation III was UGT2B4 ($p = 4.06x10^{-5}$ in tumor tissue; $p = 1.68x10^{-6}$ in normal tissue).

**Electronic medical record data**

Clinical follow-up data abstracted from electronic medical records appears in **Table 21**. Pathological staging indicated T2A to T3A (IIA to III) prostate cancer. Only one subject had invasion of the lymph nodes. No subjects experienced metastatic disease. No patient experienced biochemical recurrence, though one patient experienced persistent disease.

**Table 1. Biospecimen inventory of study sample**

| | | | Frozen tissue | | |
|---|---|---|---|---|---|
| **Patient** | **ID No.** | **Treatment** | **Normal** | **Tumor** | **Urine** |
| 1 | 07 | SOY | X | X | X |
| 2 | 34 | SOY | X | X | X |
| 3 | 36 | PLACEBO | X | X | X |
| 4 | 37 | SOY | BPH | X | X |
| 5 | 38 | PLACEBO | X | X | X |
| 6 | 50 | SOY | X | X | X |

Patient No. 4 (ID #37): normal tissue contained benign prostatic hyperplasia (BPH) cells, a benign condition.

**Table 2. Frozen prostate tissue in total inventory**

| | ID No. | Treatment | Normal | Tumor | Normal/tumor tissue pairs |
|---|---|---|---|---|---|
| 1 | 01 | PLACEBO | 0 | LYSATE | No, lysate only |
| 2 | 02 | PLACEBO | 0 | 0 | No, no samples in inventory |
| 3 | 04 | SOY | 0 | 0 | No, no samples in inventory |
| 4 | 06 | SOY | 0 | 0 | No, no samples in inventory |
| 5 | 07 | SOY | 1 | 1 | Yes |
| 6 | 08 | PLACEBO | 0 | 0 | No, no samples in inventory |
| 7 | 11 | PLACEBO | 0 | 0 | No, no samples in inventory |
| 8 | 31 | SOY | 2 | 0 | No, no normal tissue in inventory |
| 9 | 33 | PLACEBO | LYSATE | LYSATE | No, lysate only |
| 10 | 34 | SOY | 1 | 1 | Yes |
| 11 | 36 | PLACEBO | 1 | 1 | Yes |
| 12 | 37 | SOY | BPH | 1 | Yes, BPH is a benign condition |
| 13 | 38 | PLACEBO | 1 | 1 | Yes |
| 14 | 50 | SOY | 1 | 1 | Yes |

BPH, benign prostatic hyperplasia

**Table 3. Baseline characteristics of study sample**

| **Patient, No.** | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **ID No.** | 07 | 34 | 36 | 37 | 38 | 50 |
| **Treatment** | SOY | SOY | PLACEBO | SOY | PLACEBO | SOY |
| **Age, yr** | 69 | 75 | 62 | 61 | 63 | 59 |
| **Race** | White | White | White | White | White | White |
| **Ethnicity** | Non-Hispanic | Non-Hispanic | Non-Hispanic | Non-Hispanic | Non-Hispanic | Non-Hispanic |
| **Weight, lbs** | 182 | 148 | 209 | 152 | 216 | 245 |
| **Height, in** | 68 | 68 | 71 | 68 | 70 | 67 |
| **BMI, kg/m$^2$** | 27.7 | 22.5 | 29.2 | 23.1 | 30.8 | 38.4 |
| **PSA, ng/mL** | 10.9 | 5.6 | 4.1 | 21.6 | 7.3 | 9.5 |
| **Clinical stage** | T2A | T2A | T2C | T2A | T2A | T1C |
| **Gleason score** | 7 | 9 | 7 | 7 | 6 | 7 |
| **Approach** | RRP | RRP | RRP | RRP | RRP | RRP |
| **Concurrent ADT** | NO | NO | NO | YES | NO | NO |
| **Complications** | NO | NO | NO | NO | NO | NO |
| **Compliant** | YES | YES | YES | YES | YES | YES |
| **Equol producer** | YES | NO | PLACEBO | NO | PLACEBO | NO |

BMI, body mass index; PSA, prostate specific antigen; RRP, radical retropubic prostatectomy

**Table 4. Urinary isoflavone concentrations (nmol/L) of study sample**

| **Patient** | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **ID No.** | 07 | 34 | 36 | 37 | 38 | 50 |
| **Treatment** | SOY | SOY | PLACEBO | SOY | PLACEBO | SOY |
| **Genistein** | 2.551 | 5.843 | 0.112 | 1.370 | 0.029 | 12.533 |
| **Adjusted genistein** | 2.278 | 5.745 | 0.093 | 2.630 | 0.027 | ND |
| **Daidzein** | 19.091 | 44.402 | 0.291 | 12.839 | 0.042 | 98.164 |
| **Adjusted daidzein** | 17.046 | 43.660 | 0.241 | 24.642 | 0.039 | ND |
| **Glycitein** | 4.617 | 17.350 | 0.124 | 10.240 | 0.029 | ND |
| **Adjusted glycitein** | 4.122 | 17.350 | 0.124 | 10.240 | 0.029 | 22.276 |
| **ODMA** | 0.184 | 12.307 | 0.011 | 2.969 | ND | 1.158 |
| **Adjusted ODMA** | 0.164 | 12.307 | 0.009 | 5.700 | ND | ND |
| **DHD** | 6.560 | 10.252 | 0.117 | 4.835 | 0.026 | 4.577 |
| **Adjusted DHD** | 5.858 | 10.081 | 0.097 | 9.280 | 0.024 | ND |
| **Equol** | 24.073 | 0.028 | 0.011 | 0.018 | ND | ND |
| **Equol:daidzein** | 1.261 | 0.001 | 0.039 | 0.001 | ND | ND |
| **Equol producer** | YES | NO | PLACEBO | NO | PLACEBO | NO |
| **Creatinine (mg/dL)** | 112 | 101.7 | 120.6 | 52.1 | 107.8 | ND |

ND, no data; ODMA, O-desmethylangolensin; DHD, dihydrodaizein

**Table 5.** Differentially expressed genes (FDR < 0.05) down-regulated by soy isoflavone supplementation of the 128 differentially expressed genes from tumor tissue obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ENSG00000112175 | BMP5 | Bone morphogenetic protein 5 | 6 | 55618443 | 55740362 | 234 | 6.66E-16 | 2.79E-11 |
| 2 | ENSG00000170290 | SLN | Sarcolipin | 11 | 107578104 | 107590419 | 269 | 2.37E-14 | 4.72E-10 |
| 3 | ENSG00000156234 | CXCL13 | Chemokine (C-X-C motif) ligand 13 | 4 | 78432907 | 78532988 | 129 | 3.39E-14 | 4.72E-10 |
| 4 | ENSG00000254851 | RP11-109L13.1 | | 11 | 117006244 | 117009298 | 340 | 4.88E-14 | 5.11E-10 |
| 5 | ENSG00000181617 | FDCSP | Follicular dendritic cell secreted protein | 4 | 71091788 | 71100969 | 104 | 3.68E-11 | 2.74E-07 |
| 6 | ENSG00000145681 | HAPLN1 | Hyaluronan and proteoglycan link protein 1 | 5 | 82933624 | 83017432 | 60 | 3.93E-11 | 2.74E-07 |
| 7 | ENSG00000131094 | C1QL1 | Complement component 1, q subcomponent-like 1 | 17 | 43037061 | 43045439 | 49 | 2.15E-10 | 1.12E-06 |
| 8 | ENSG00000237250 | RP11-193H5.1 | | 1 | 238025475 | 238091621 | 350 | 6.88E-10 | 3.20E-06 |
| 9 | ENSG00000171201 | SMR3B | Submaxillary gland androgen regulated protein 3B | 4 | 71235810 | 71255961 | 67 | 1.73E-09 | 7.24E-06 |
| 10 | ENSG00000164879 | CA3 | Carbonic anhydrase III, muscle specific | 8 | 86285665 | 86361269 | 35 | 3.96E-09 | 1.51E-05 |
| 11 | ENSG00000075043 | KCNQ2 | Potassium voltage-gated channel, KQT-like subfamily, member 2 | 20 | 62037542 | 62103993 | 32 | 5.51E-09 | 1.92E-05 |
| 12 | ENSG00000101204 | CHRNA4 | Cholinergic receptor, nicotinic, alpha 4 (neuronal) | 20 | 61975420 | 62009753 | 56 | 7.95E-09 | 2.56E-05 |
| 13 | ENSG00000184374 | COLEC10 | Collectin sub-family member 10 (C-type lectin) | 8 | 120007691 | 120118821 | 40 | 9.49E-09 | 2.84E-05 |
| 14 | ENSG00000171446 | KRT27 | Keratin 27 | 17 | 38933060 | 38938786 | 60 | 1.87E-08 | 5.21E-05 |
| 15 | ENSG00000258027 | RP11-597A11.1 | | 14 | 20078033 | 20146082 | 31 | 2.20E-08 | 5.76E-05 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | ENSG00000117322 | CR2 | Complement component (3d/Epstein Barr virus) receptor 2 | 1 | 207627575 | 207663240 | 29 | 2.54E-08 | 6.25E-05 |
| 17 | ENSG00000185742 | C11orf87 | Chromosome 11 open reading frame 87 | 11 | 109292846 | 109299840 | 58 | 3.47E-08 | 8.04E-05 |
| 18 | ENSG00000197616 | MYH6 | Myosin, heavy chain 6, cardiac muscle, alpha | 14 | 23851199 | 23877486 | 28 | 3.68E-08 | 8.04E-05 |
| 19 | ENSG00000164616 | FBXL21 | F-box and leucine-rich repeat protein 21 | 5 | 135266006 | 135287280 | 28 | 3.84E-08 | 8.04E-05 |
| 20 | ENSG00000121075 | TBX4 | T-box 4 | 17 | 59529765 | 59562471 | 23 | 8.97E-08 | 0.00017071 |
| 21 | ENSG00000253400 | RP11-337A23.6 | | 9 | 26801731 | 26805860 | 181 | 1.13E-07 | 0.00020532 |
| 22 | ENSG00000253998 | IGKV2-29 | Immunoglobulin kappa variable 2-29 | 2 | 89533655 | 89534393 | 174 | 1.35E-07 | 0.00023415 |
| 23 | ENSG00000266554 | RP11-537H14.2 | | 18 | 14946266 | 14973769 | 78 | 1.40E-07 | 0.00023415 |
| 24 | ENSG00000185668 | POU3F1 | POU class 3 homeobox 1 | 1 | 38509523 | 38512450 | 23 | 1.53E-07 | 0.00024649 |
| 25 | ENSG00000184613 | NELL2 | NEL-like 2 (chicken) | 12 | 44902058 | 45315631 | 19 | 3.99E-07 | 0.0005937 |
| 26 | ENSG00000184258 | CDR1 | Cerebellar degeneration-related protein 1, 34kDa | X | 139865425 | 139866723 | 67 | 4.59E-07 | 0.00062761 |
| 27 | ENSG00000188778 | ADRB3 | Adrenoceptor beta 3 | 8 | 37820516 | 37824483 | 22 | 4.65E-07 | 0.00062761 |
| 28 | ENSG00000104921 | FCER2 | Fc fragment of IgE, low affinity II, receptor for (CD23) | 19 | 7753644 | 7767032 | 22 | 5.60E-07 | 0.00072277 |
| 29 | ENSG00000257357 | RP11-244H18.3 | | 14 | 20105463 | 20109540 | 24 | 5.87E-07 | 0.00072277 |
| 30 | ENSG00000130600 | H19 | H19, imprinted maternally expressed transcript (non-protein coding) | 11 | 2016406 | 2022700 | 18 | 6.06E-07 | 0.00072277 |
| 31 | ENSG00000257432 | RP11-244H18.4 | | 14 | 20097282 | 20098616 | 22 | 6.22E-07 | 0.00072277 |
| 32 | ENSG00000224521 | RP11-438F14.3 | | 1 | 248712057 | 248727140 | 134 | 9.94E-07 | 0.00109547 |
| 33 | ENSG00000102683 | SGCG | Sarcoglycan, gamma (35kDa dystrophin-associated glycoprotein) | 13 | 23755091 | 23899304 | 17 | 1.06E-06 | 0.00113499 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 34 | ENSG00000141744 | PNMT | Phenylethanolamine N-methyltransferase | 17 | 37824234 | 37826728 | 17 | 1.19E-06 | 0.00124436 |
| 35 | ENSG00000138207 | RBP4 | Retinol binding protein 4, plasma | 10 | 95351444 | 95361501 | 16 | 1.46E-06 | 0.00145278 |
| 36 | ENSG00000263556 | RN7SL383P | RNA, 7SL, cytoplasmic 383, pseudogene | 5 | 44716292 | 44716587 | 130 | 1.57E-06 | 0.00153224 |
| 37 | ENSG00000179455 | MKRN3 | Makorin ring finger protein 3 | 15 | 23810454 | 23873064 | 23 | 1.73E-06 | 0.00164774 |
| 38 | ENSG00000181072 | CHRM2 | Cholinergic receptor, muscarinic 2 | 7 | 136553416 | 136705002 | 33 | 1.83E-06 | 0.00170513 |
| 39 | ENSG00000260125 | RP11-31E22.1 | | 15 | 86838880 | 86860404 | 40 | 2.03E-06 | 0.0018495 |
| 40 | ENSG00000255399 | TBX5-AS1 | TBX5 antisense RNA 1 | 12 | 114845996 | 114850636 | 16 | 2.42E-06 | 0.00215714 |
| 41 | ENSG00000170054 | SERPINA9 | Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase | 14 | 94929054 | 94946026 | 118 | 2.55E-06 | 0.00218999 |
| 42 | ENSG00000019169 | MARCO | Macrophage receptor with collagenous structure | 2 | 119699742 | 119752236 | 15 | 2.62E-06 | 0.00219449 |
| 43 | ENSG00000100427 | MLC1 | Megalencephalic leukoencephalopathy with subcortical cysts 1 | 22 | 50497820 | 50524331 | 14 | 3.78E-06 | 0.00304331 |
| 44 | ENSG00000162989 | KCNJ3 | Potassium inwardly-rectifying channel, subfamily J, member 3 | 2 | 155554811 | 155714863 | 14 | 4.62E-06 | 0.00360292 |
| 45 | ENSG00000118231 | CRYGD | Crystallin, gamma D | 2 | 208986331 | 208989225 | 49 | 4.79E-06 | 0.00364794 |
| 46 | ENSG00000173976 | RAX2 | Retina and anterior neural fold homeobox 2 | 19 | 3769087 | 3772233 | 109 | 5.56E-06 | 0.00408458 |
| 47 | ENSG00000228408 | RP1-111D6.3 | | 6 | 149539062 | 149565208 | 106 | 5.56E-06 | 0.00408458 |
| 48 | ENSG00000249201 | CTD-3080P12.3 | | 5 | 1173256 | 1178720 | 102 | 7.32E-06 | 0.00504856 |
| 49 | ENSG00000185002 | RFX6 | Regulatory factor X, 6 | 6 | 117198375 | 117253326 | 15 | 7.36E-06 | 0.00504856 |
| 50 | ENSG00000105989 | WNT2 | Wingless-type MMTV integration site family member 2 | 7 | 116916685 | 116963343 | 12 | 9.60E-06 | 0.00648123 |
| 51 | ENSG00000261076 | RP11-179B15.6 | | 10 | 66085374 | 60086790 | 43 | 1.14E-05 | 0.00732234 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 52 | ENSG00000228294 | AL589743.2 | | 14 | 19670480 | 19686002 | 26 | 1.23E-05 | 0.00777982 |
| 53 | ENSG00000257162 | RP11-244H18.2 | | 14 | 20071068 | 20074896 | 93 | 1.30E-05 | 0.00814965 |
| 54 | ENSG00000234184 | RP5-887A10.1 | | 1 | 81001440 | 81112473 | 14 | 1.36E-05 | 0.00839273 |
| 55 | ENSG00000181195 | PENK | Proenkephalin | 8 | 57349233 | 57359293 | 12 | 1.51E-05 | 0.00916479 |
| 56 | ENSG00000235743 | RP11-439H9.1 | | 6 | 23337939 | 23346788 | 92 | 1.77E-05 | 0.01026902 |
| 57 | ENSG00000238773 | AL137855.1 | | 1 | 58222756 | 58222848 | 92 | 1.77E-05 | 0.01026902 |
| 58 | ENSG00000211892 | IGHG4 | Immunoglobulin heavy constant gamma 4 (G4m marker) | 14 | 106090687 | 106092403 | 11 | 2.08E-05 | 0.0118659 |
| 59 | ENSG00000188176 | SMTNL2 | Smoothelin-like 2 | 17 | 4487294 | 4511614 | 12 | 2.10E-05 | 0.0118659 |
| 60 | ENSG00000211670 | IGLV3-9 | Immunoglobulin lambda variable 3-9 | 22 | 23161507 | 23162253 | 12 | 2.30E-05 | 0.01283791 |
| 61 | ENSG00000174482 | LINGO2 | Leucine rich repeat and Ig domain containing 2 | 9 | 27948076 | 28670283 | 12 | 2.65E-05 | 0.01439979 |
| 62 | ENSG00000123572 | NRK | Nik related kinase | X | 105066536 | 105202602 | 10 | 3.94E-05 | 0.02086231 |
| 63 | ENSG00000188000 | OR7D2 | Olfactory receptor, family 7, subfamily D, member 2 | 19 | 9296279 | 9299493 | 14 | 4.09E-05 | 0.02088368 |
| 64 | ENSG00000139767 | SRRM4 | Serine/arginine repetitive matrix 4 | 12 | 119419300 | 119600856 | 14 | 4.20E-05 | 0.02116363 |
| 65 | ENSG00000171189 | GRIK1 | Glutamate receptor, ionotropic, kainate 1 | 21 | 30909254 | 31312351 | 10 | 4.36E-05 | 0.021459 |
| 66 | ENSG00000149295 | DRD2 | Dopamine receptor D2 | 11 | 113280318 | 113346413 | 10 | 4.60E-05 | 0.02179889 |
| 67 | ENSG00000260412 | RP11-438B23.2 | | 9 | 27937615 | 27944495 | 15 | 5.25E-05 | 0.02439748 |
| 68 | ENSG00000259384 | GH1 | Growth hormone 1 | 17 | 61994560 | 61996179 | 25 | 5.68E-05 | 0.02585888 |
| 69 | ENSG00000234665 | RP11-262H14.3 | | 9 | 66513488 | 66553911 | 14 | 5.86E-05 | 0.0263856 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 70 | ENSG00000255760 | RP11-428G5.5 | | 12 | 32030013 | 32040137 | 13 | 5.99E-05 | 0.02656543 |
| 71 | ENSG00000130700 | GATA5 | GATA binding protein 5 | 20 | 61038553 | 61051026 | 10 | 6.08E-05 | 0.02656543 |
| 72 | ENSG00000154016 | GRAP | GRB2-related adaptor protein | 17 | 18923986 | 18950950 | 74 | 6.66E-05 | 0.02819966 |
| 73 | ENSG00000186207 | LCE5A | Late cornified envelope 5A | 1 | 152483320 | 152484653 | 71 | 6.66E-05 | 0.02819966 |
| 74 | ENSG00000163534 | FCRL1 | Fc receptor-like 1 | 1 | 157764193 | 157789895 | 10 | 6.67E-05 | 0.02819966 |
| 75 | ENSG00000101440 | ASIP | Agouti signaling protein | 20 | 32782375 | 32857150 | 10 | 7.24E-05 | 0.03002326 |
| 76 | ENSG00000234931 | MARK2P15 | MAP/microtubule affinity-regulating kinase 2 pseudogene 15 | 10 | 85071384 | 85074231 | 29 | 7.24E-05 | 0.03002326 |
| 77 | ENSG00000234224 | TMEM229A | Transmembrane protein 229A | 7 | 123670973 | 123673523 | 11 | 7.46E-05 | 0.0300731 |
| 78 | ENSG00000175513 | TSGA10IP | Testis specific, 10 interacting protein | 11 | 65712916 | 65727434 | 13 | 7.54E-05 | 0.0300731 |
| 79 | ENSG00000233403 | RP11-86A5.1 | | X | 25896456 | 25911661 | 31 | 8.60E-05 | 0.03387416 |
| 80 | ENSG00000254278 | RP11-278I4.2 | | 8 | 120075181 | 120081021 | 71 | 9.59E-05 | 0.03616201 |
| 81 | ENSG00000231882 | F10-AS1 | F10 antisense RNA 1 | 13 | 113782469 | 113783194 | 67 | 9.59E-05 | 0.03616201 |
| 82 | ENSG00000229231 | FEM1AP1 | Fem-1 homolog a (C. elegans) pseudogene 1 | 21 | 15134659 | 15136653 | 67 | 9.59E-05 | 0.03616201 |
| 83 | ENSG00000197766 | CFD | Complement factor D (adipsin) | 19 | 859453 | 863569 | 9 | 9.99E-05 | 0.03698971 |
| 84 | ENSG00000100433 | KCNK10 | Potassium channel, subfamily K, member 10 | 14 | 88649113 | 88793251 | 13 | 0.00010074 | 0.03698971 |
| 85 | ENSG00000170356 | OR2A20P | Olfactory receptor, family 2, subfamily A, member 20 pseudogene | 7 | 143947138 | 143950050 | 12 | 0.00010074 | 0.03698971 |
| 86 | ENSG00000129988 | LBP | Lipopolysaccharide binding protein | 20 | 36974759 | 37005665 | 11 | 0.00010695 | 0.038504 |
| 87 | ENSG00000213424 | KRT222 | Keratin 222 | 17 | 38810917 | 38821433 | 10 | 0.00010762 | 0.038504 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 88 | ENSG00000260896 | RP11-314O13.1 | | 16 | 80862632 | 80926492 | 9 | 0.00011354 | 0.04027704 |
| 89 | ENSG00000230229 | RP11-90J7.4 | | 10 | 80112354 | 80115857 | 14 | 0.00012331 | 0.04266026 |
| 90 | ENSG00000110675 | ELMOD1 | ELMO/CED-12 domain containing 1 | 11 | 107461817 | 107537505 | 9 | 0.00012445 | 0.04270224 |
| 91 | ENSG00000121270 | ABCC11 | ATP-binding cassette, subfamily C (CFTR/MRP), member 11 | 16 | 48200821 | 48281479 | 9 | 0.00013328 | 0.04499134 |
| 92 | ENSG00000259761 | RP11-138H10.2 | | 15 | 87585285 | 87590392 | 64 | 0.00014028 | 0.04660497 |
| 93 | ENSG00000111704 | NANOG | NANOG homeobox | 12 | 7940390 | 7948655 | 62 | 0.00014028 | 0.04660497 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 6. Differentially expressed genes (FDR < 0.05) up-regulated by soy isoflavone supplementation of the 128 differentially expressed genes from tumor tissue obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)**

| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ENSG00000164266 | SPINK1 | Serine peptidase inhibitor, Kazal type 1 | 5 | 147204131 | 147211349 | 518 | 9.04E-11 | 5.41E-07 |
| 2 | ENSG00000052850 | ALX4 | ALX homeobox 4 | 11 | 44281994 | 44331716 | 131 | 5.59E-08 | 0.0001115 |
| 3 | ENSG00000171564 | FGB | Fibrinogen beta chain | 4 | 155484108 | 155492238 | 222 | 2.78E-07 | 0.00043134 |
| 4 | ENSG00000204612 | FOXB2 | Forkhead box B2 | 9 | 79634571 | 79635869 | 662 | 4.11E-07 | 0.0005937 |
| 5 | ENSG00000233760 | AC004947.2 | | 7 | 26591441 | 26596819 | 68 | 5.90E-07 | 0.00072277 |
| 6 | ENSG00000144355 | DLX1 | Distal-less homeobox 1 | 2 | 172949468 | 172954405 | 66 | 7.26E-07 | 0.00082111 |
| 7 | ENSG00000007306 | CEACAM7 | Carcinoembryonic antigen-related cell adhesion molecule 7 | 19 | 42177235 | 42210895 | 63 | 1.42E-06 | 0.00145162 |
| 8 | ENSG00000170369 | CST2 | Cystatin SA | 20 | 23804406 | 23807368 | 45 | 2.56E-06 | 0.00218999 |
| 9 | ENSG00000096006 | CRISP3 | Cysteine-rich secretory protein 3 | 6 | 49695097 | 49712150 | 44 | 3.69E-06 | 0.00302595 |
| 10 | ENSG00000226278 | PSPHP1 | Phosphoserine phosphatase pseudogene 1 | 7 | 55832490 | 55840981 | 116 | 4.65E-06 | 0.00360292 |
| 11 | ENSG00000171759 | PAH | Phenylalanine hydroxylase | 12 | 103230663 | 103352188 | 36 | 6.65E-06 | 0.00479909 |
| 12 | ENSG00000126733 | DACH2 | Dachshund homolog 2 (Drosophila) | X | 85403462 | 86087607 | 36 | 7.24E-06 | 0.00504856 |
| 13 | ENSG00000240045 | RP11-451G4.2 | | 3 | 155008018 | 155011564 | 48 | 1.02E-05 | 0.00680086 |
| 14 | ENSG00000227560 | RP11-139K1.2 | | 10 | 114747235 | 114747624 | 46 | 1.12E-05 | 0.00732234 |
| 15 | ENSG00000170373 | CST1 | Cystatin SN | 20 | 23728190 | 23731905 | 29 | 1.67E-05 | 0.00996704 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | ENSG00000153294 | GPR115 | G protein-coupled receptor 115 | 6 | 47653600 | 47689757 | 40 | 2.42E-05 | 0.01331627 |
| 17 | ENSG00000254349 | RP11-758M4.1 | | 8 | 75512010 | 75735548 | 29 | 3.75E-05 | 0.02015023 |
| 18 | ENSG00000156096 | UGT2B4 | UDP glucuronosyltransferase 2 family, polypeptide B4 | 4 | 70345883 | 70391732 | 28 | 4.06E-05 | 0.02088368 |
| 19 | ENSG00000134443 | GRP | Gastrin-releasing peptide | 18 | 56887400 | 56898006 | 25 | 4.08E-05 | 0.02088368 |
| 20 | ENSG00000171560 | FGA | Fibrinogen alpha chain | 4 | 155504278 | 155511918 | 225 | 4.28E-05 | 0.02134795 |
| 21 | ENSG00000100665 | SERPINA4 | Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, | 14 | 95027428 | 95036250 | 63 | 4.48E-05 | 0.02179889 |
| 22 | ENSG00000230327 | AC009234.1 | | 2 | 50815828 | 50816553 | 39 | 4.60E-05 | 0.02179889 |
| 23 | ENSG00000172238 | ATOH1 | Atonal homolog 1 (Drosophila) | 4 | 94750042 | 94751221 | 216 | 4.63E-05 | 0.02179889 |
| 24 | ENSG00000170426 | SDR9C7 | Short chain dehydrogenase/reductase family 9C, member 7 | 12 | 57316938 | 57328189 | 25 | 5.60E-05 | 0.02573882 |
| 25 | ENSG00000154975 | CA10 | Carbonic anhydrase X | 17 | 49707674 | 50237377 | 36 | 6.09E-05 | 0.02656543 |
| 26 | ENSG00000237567 | RP3-359N14.2 | | 6 | 106060542 | 106080071 | 24 | 7.39E-05 | 0.0300731 |
| 27 | ENSG00000095627 | TDRD1 | Tudor domain containing 1 | 10 | 115939029 | 115992063 | 23 | 7.52E-05 | 0.0300731 |
| 28 | ENSG00000224099 | AC064834.1 | | 2 | 196313256 | 196343649 | 31 | 8.66E-05 | 0.03387416 |
| 29 | ENSG00000146039 | SLC17A4 | Solute carrier family 17 (sodium phosphate), | 6 | 25754927 | 25781419 | 27 | 8.89E-05 | 0.03447194 |
| 30 | ENSG00000261045 | RP11-673P17.4 | | 16 | 27169775 | 27187908 | 22 | 0.00010714 | 0.038504 |
| 31 | ENSG00000247627 | MTND4P12 | MT-ND4 pseudogene 12 | 5 | 134262350 | 134263726 | 18 | 0.00011453 | 0.04028588 |
| 32 | ENSG00000243236 | RP1-214M20.2 | | 6 | 52804524 | 52822319 | 18 | 0.00011768 | 0.04105023 |
| 33 | ENSG00000156925 | ZIC3 | ZIC family member 3 | X | 136648301 | 136659850 | 53 | 0.00012932 | 0.04401105 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 34 | ENSG00000128564 | VGF | VGF nerve growth factor inducible | 7 | 100805790 | 100808874 | 19 | 0.00014537 | 0.04791442 |
| 35 | ENSG00000187416 | LHFPL3 | Lipoma HMGIC fusion partner-like 3 | 7 | 103969104 | 104549001 | 21 | 0.00015021 | 0.04912182 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 7. Differentially expressed genes (FDR < 0.05) down-regulated by soy isoflavone supplementation of the 108 differentially expressed genes from only tumor tissue obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)**

| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ENSG00000112175 | BMP5 | Bone morphogenetic protein 5 | 6 | 55618443 | 55740362 | 234 | 6.66E-16 | 2.79E-11 |
| 2 | ENSG00000170290 | SLN | Sarcolipin | 11 | 1.08E+08 | 1.08E+08 | 269 | 2.37E-14 | 4.72E-10 |
| 3 | ENSG00000156234 | CXCL13 | Chemokine (C-X-C motif) ligand 13 | 4 | 78432907 | 78532988 | 129 | 3.39E-14 | 4.72E-10 |
| 4 | ENSG00000181617 | FDCSP | Follicular dendritic cell secreted protein | 4 | 71091788 | 71100969 | 104 | 3.68E-11 | 2.74E-07 |
| 5 | ENSG00000145681 | HAPLN1 | Hyaluronan and proteoglycan link protien 1 | 5 | 82933624 | 83017432 | 60 | 3.93E-11 | 2.74E-07 |
| 6 | ENSG00000131094 | C1QL1 | Complement component 1, q subcomponent-like 1 | 17 | 43037061 | 43045439 | 49 | 2.15E-10 | 1.12E-06 |
| 7 | ENSG00000237250 | RP11-193H5.1 | | 1 | 238025475 | 238091621 | 350 | 6.88E-10 | 3.20E-06 |
| 8 | ENSG00000171201 | SMR3B | Submaxillary gland androgen regulated protein 3B | 4 | 71235810 | 71255961 | 67 | 1.73E-09 | 7.24E-06 |
| 9 | ENSG00000164879 | CA3 | Carbonic anhydrase III, muscle specific | 8 | 86285665 | 86361269 | 35 | 3.96E-09 | 1.51E-05 |
| 10 | ENSG00000075043 | KCNQ2 | Potassium voltage-gated channel, KQT-like subfamily | 20 | 62037542 | 62103993 | 32 | 5.51E-09 | 1.92E-05 |
| 11 | ENSG00000101204 | CHRNA4 | Cholinergic receptor, nicotinic, alpha 4 (neuronal) | 20 | 61975420 | 62009753 | 56 | 7.95E-09 | 2.56E-05 |
| 12 | ENSG00000184374 | COLEC10 | Collectin subfamily member 10 | 8 | 120007691 | 120118821 | 40 | 9.49E-09 | 2.84E-05 |
| 13 | ENSG00000171446 | KRT27 | Keratin 27 | 17 | 38933060 | 38938786 | 60 | 1.87E-08 | 5.21E-05 |
| 14 | ENSG00000117322 | CR2 | Complement component (3d/Epstein Barr virus) receptor 2 | 1 | 207627575 | 207663240 | 29 | 2.54E-08 | 6.25E-05 |
| 15 | ENSG00000185742 | C11orf87 | Chromosome 11 open reading frame 87 | 11 | 109292846 | 109299840 | 58 | 3.47E-08 | 8.04E-05 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | ENSG00000197616 | MYH6 | Myosin, heavy chain 6, cardiac muscle, alpha | 14 | 23851199 | 23877486 | 28 | 3.68E-08 | 8.04E-05 |
| 17 | ENSG00000164616 | FBXL21 | F-box and leucine-rich repeat protein 21 | 5 | 135266006 | 135287820 | 28 | 3.84E-08 | 8.04E-05 |
| 18 | ENSG00000253400 | RP11-337A23.6 | | 9 | 26801731 | 26805860 | 181 | 1.13E-07 | 0.000205318 |
| 19 | ENSG00000266554 | RP11-527H14.2 | | 18 | 14946266 | 14973769 | 78 | 1.40E-07 | 0.000234152 |
| 20 | ENSG00000185668 | POU3F1 | POU class 3 homeobox 1 | 1 | 38509523 | 38512450 | 23 | 1.53E-07 | 0.000246492 |
| 21 | ENSG00000184613 | NELL2 | NEL-like 2 (chicken) | 12 | 44902058 | 45315631 | 19 | 3.99E-07 | 0.000593697 |
| 22 | ENSG00000184258 | CDR1 | Cerebellar degeneration-related protein 1, 34kDa | X | 139865425 | 139866723 | 67 | 4.59E-07 | 0.000627607 |
| 23 | ENSG00000188778 | ADRB3 | Adrenoreceptor beta 3 | 8 | 37820516 | 37824483 | 22 | 4.65E-07 | 0.000627607 |
| 24 | ENSG00000104921 | FCER2 | Fc fragment of IgE, low affinity II, receptor for (CD23) | 19 | 7753644 | 7767032 | 22 | 5.60E-07 | 0.00072277 |
| 25 | ENSG00000130600 | H19 | H19, imprinted maternally expressed transcript (non- | 11 | 2016406 | 2022700 | 18 | 6.06E-07 | 0.00072277 |
| 26 | ENSG00000257432 | RP11-244H18.4 | | 14 | 20097282 | 20098616 | 22 | 6.22E-07 | 0.00072277 |
| 27 | ENSG00000224521 | RP11-438F14.3 | | 1 | 248712057 | 248727140 | 134 | 9.94E-07 | 0.001095466 |
| 28 | ENSG00000102683 | SGCG | Sarcoglycan, gamma (35kDa dystrophin-associated | 13 | 23755091 | 23899304 | 17 | 1.06E-06 | 0.001134986 |
| 29 | ENSG00000141744 | PNMT | Phenylethanolamine N-methyltransferase | 17 | 37824234 | 37826728 | 17 | 1.19E-06 | 0.001244355 |
| 30 | ENSG00000138207 | RBP4 | Retinol binding protein 4, plasma | 10 | 95351444 | 95361501 | 16 | 1.46E-06 | 0.00145278 |
| 31 | ENSG00000263556 | RN7SL383P | RNA, 7SL, cytoplasmic 383, pseudogene | 5 | 44716292 | 44716587 | 130 | 1.57E-06 | 0.001532245 |
| 32 | ENSG00000179455 | MKRN3 | Makorin ring finger protein 3 | 15 | 23810454 | 23873064 | 23 | 1.73E-06 | 0.00164774 |
| 33 | ENSG00000181072 | CHRM2 | Cholinergic receptor, muscarinic 2 | 7 | 136553416 | 136705002 | 33 | 1.83E-06 | 0.001705134 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 34 | ENSG00000255399 | TBX5-AS1 | TBX5 antisense RNA 1 | 12 | 114845996 | 114850636 | 16 | 2.42E-06 | 0.002157141 |
| 35 | ENSG00000170054 | SERPINA9 | Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase | 14 | 94929054 | 94946026 | 118 | 2.55E-06 | 0.002189989 |
| 36 | ENSG00000019169 | MARCO | Macrophage receptor with collagenase structure | 2 | 119699742 | 119752236 | 15 | 2.62E-06 | 0.002194493 |
| 37 | ENSG00000100427 | MLC1 | Megalencephalic leukoencephalopathy with subcortical cysts | 22 | 50497820 | 50524331 | 14 | 3.78E-06 | 0.003043306 |
| 38 | ENSG00000162989 | KCNJ3 | Potassium inwardly-rectifying channel | 2 | 155554811 | 155714863 | 14 | 4.62E-06 | 0.003602916 |
| 39 | ENSG00000118231 | CRYGD | Crystallin, gamma D | 2 | 208986331 | 208989225 | 49 | 4.79E-06 | 0.003647939 |
| 40 | ENSG00000173976 | RAX2 | Retina and anterior neural fold homeobox 2 | 19 | 3769087 | 3772233 | 109 | 5.56E-06 | 0.00408458 |
| 41 | ENSG00000228408 | RP1-111D6.3 | | 6 | 149539062 | 149565208 | 106 | 5.56E-06 | 0.00408458 |
| 42 | ENSG00000249201 | CTD-3080P12.3 | | 5 | 1173256 | 1178720 | 102 | 7.32E-06 | 0.005048563 |
| 43 | ENSG00000185002 | RFX6 | Regulatory factor X, 6 | 6 | 117198375 | 117253326 | 15 | 7.36E-06 | 0.005048563 |
| 44 | ENSG00000105989 | WNT2 | Wingless-type MMTV integration site family member 2 | 7 | 116916685 | 116963343 | 12 | 9.60E-06 | 0.006481232 |
| 45 | ENSG00000261076 | RP11-179B15.6 | | 10 | 66085374 | 60086790 | 43 | 1.14E-05 | 0.007322341 |
| 46 | ENSG00000228294 | AL589743.2 | | 14 | 19670480 | 19686002 | 26 | 1.23E-05 | 0.00777982 |
| 47 | ENSG00000257162 | RP11-244H18.2 | | 14 | 20071068 | 20074896 | 93 | 1.30E-05 | 0.008149649 |
| 48 | ENSG00000234184 | RP5-887A10.1 | | 1 | 81001440 | 81112473 | 14 | 1.36E-05 | 0.008392733 |
| 49 | ENSG00000181195 | PENK | Proenkephalin | 8 | 57349233 | 57359293 | 12 | 1.51E-05 | 0.009164791 |
| 50 | ENSG00000235743 | RP11-439H9.1 | | 6 | 23337939 | 23346788 | 92 | 1.77E-05 | 0.010269018 |
| 51 | ENSG00000238773 | AL137855.1 | | 1 | 58222756 | 58222848 | 92 | 1.77E-05 | 0.010269018 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 52 | ENSG00000211892 | IGHG4 | Immunoglobulin heavy constant gamma 4 (G4m marker) | 14 | 106090687 | 106092403 | 11 | 2.08E-05 | 0.011865899 |
| 53 | ENSG00000188176 | SMTNL2 | Smoothelin-like 2 | 17 | 4487294 | 4511614 | 12 | 2.10E-05 | 0.011865899 |
| 54 | ENSG00000211670 | IGLV3-9 | Immunoglobulin lambda variable 3-9 (gene/pseudoge | 22 | 23161507 | 23162253 | 12 | 2.30E-05 | 0.012837914 |
| 55 | ENSG00000174482 | LINGO2 | Leucine rich repeat and Ig domain containing 2 | 9 | 27948076 | 28670283 | 12 | 2.65E-05 | 0.014399793 |
| 56 | ENSG00000123572 | NRK | NIK related kinase | X | 105066536 | 105202602 | 10 | 3.94E-05 | 0.020862314 |
| 57 | ENSG00000139767 | SRRM4 | Serine/arginine repetitive matrix 4 | 12 | 119419300 | 119600856 | 14 | 4.20E-05 | 0.021163626 |
| 58 | ENSG00000171189 | GRIK1 | Glutamate receptor, ionotropic, kainate 1 | 21 | 30909254 | 31312351 | 10 | 4.36E-05 | 0.021458998 |
| 59 | ENSG00000149295 | DRD2 | Dopamine receptor D2 | 11 | 113280318 | 113346413 | 10 | 4.60E-05 | 0.021798893 |
| 60 | ENSG00000260412 | RP11-438B23.2 | | 9 | 27937615 | 27944495 | 15 | 5.25E-05 | 0.024397483 |
| 61 | ENSG00000259384 | GH1 | Growth hormone 1 | 17 | 61994560 | 61996179 | 25 | 5.68E-05 | 0.025858877 |
| 62 | ENSG00000255760 | RP11-428G5.5 | | 12 | 32030013 | 32040137 | 13 | 5.99E-05 | 0.02656543 |
| 63 | ENSG00000130700 | GATA5 | GATA binding protein 5 | 20 | 61038553 | 61051026 | 10 | 6.08E-05 | 0.02656543 |
| 64 | ENSG00000154016 | GRAP | GRB2-related adaptor protein | 17 | 18923986 | 18950950 | 74 | 6.66E-05 | 0.028199661 |
| 65 | ENSG00000186207 | LCE5A | Late cornified envelope 5A | 1 | 152483320 | 152484653 | 71 | 6.66E-05 | 0.028199661 |
| 66 | ENSG00000163534 | FCRL1 | Fc receptor-like 1 | 1 | 157764193 | 157789895 | 10 | 6.67E-05 | 0.028199661 |
| 67 | ENSG00000101440 | ASIP | Agouti signaling protein | 20 | 32782375 | 32857150 | 10 | 7.24E-05 | 0.030023263 |
| 68 | ENSG00000234224 | TMEM229A | Transmembrane protein 229A | 7 | 123670973 | 123673523 | 11 | 7.46E-05 | 0.030073102 |
| 69 | ENSG00000175513 | TSGA10IP | Testis specific, 10 interacting protein | 11 | 65712916 | 65727434 | 13 | 7.54E-05 | 0.030073102 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 70 | ENSG00000233403 | RP11-86A5.1 | | X | 25896456 | 25911661 | 31 | 8.60E-05 | 0.033874159 |
| 71 | ENSG00000254278 | RP11-278I4.2 | | 8 | 120075181 | 120081021 | 71 | 9.59E-05 | 0.036162015 |
| 72 | ENSG00000231882 | F10-As1 | F10 antisense RNA 1 | 13 | 113782469 | 113783194 | 67 | 9.59E-05 | 0.036162015 |
| 73 | ENSG00000229231 | FEM1AP1 | Fem-1 homolog a (C. elegans) pseudogene 1 | 21 | 15134659 | 15136653 | 67 | 9.59E-05 | 0.036162015 |
| 74 | ENSG00000197766 | CFD | Complement factor D (adipsin) | 19 | 859453 | 863569 | 9 | 9.99E-05 | 0.03698971 |
| 75 | ENSG00000100433 | KCNK10 | Potassium channel, subfamily K, member 10 | 14 | 88649113 | 88793251 | 13 | 0.000100736 | 0.03698971 |
| 76 | ENSG00000129988 | LBP | Lipopolysaccharide binding protein | 20 | 36974759 | 37005665 | 11 | 0.000106947 | 0.038504 |
| 77 | ENSG00000213424 | KRT222 | Keratin 222 | 17 | 38810917 | 38821433 | 10 | 0.00010762 | 0.038504 |
| 78 | ENSG00000260896 | RP11-314O13.1 | | 16 | 80862632 | 80926492 | 9 | 0.000113538 | 0.040277045 |
| 79 | ENSG00000230229 | RP11-90J7.4 | | 10 | 80112354 | 80115857 | 14 | 0.000123313 | 0.04266026 |
| 80 | ENSG00000110675 | ELMOD1 | ELMO/CED-12 domain containing 1 | 11 | 107461817 | 107537505 | 9 | 0.000124455 | 0.04270224 |
| 81 | ENSG00000121270 | ABCC11 | ATP-binding cassette, subfamily C (CFTR/MRP) | 16 | 48200821 | 48281479 | 9 | 0.000133276 | 0.044991335 |
| 82 | ENSG00000111704 | NANOG | NANOG homeobox | 12 | 7940390 | 7948655 | 62 | 0.000140283 | 0.046604968 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 8.** Differentially expressed genes (FDR < 0.05) up-regulated by soy isoflavone supplementation of the 108 differentially expressed genes from only tumor tissue obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ENSG00000052850 | ALX4 | ALX homeobox 4 | 11 | 44281994 | 44331716 | 131 | 5.59E-08 | 0.000111501 |
| 2 | ENSG00000204612 | FOXB2 | Forkhead box B2 | 9 | 79634571 | 79635869 | 662 | 4.11E-07 | 0.000593697 |
| 3 | ENSG00000144355 | DLX1 | Distal-less homeobox 1 | 2 | 172949468 | 172954405 | 66 | 7.26E-07 | 0.000821114 |
| 4 | ENSG00000007306 | CEACAM7 | Carcinoembryonic antigen-related cell adhesion molecule 7 | 19 | 42177235 | 42210895 | 63 | 1.42E-06 | 0.001451622 |
| 5 | ENSG00000171759 | PAH | Phenylalanine hydroxlyase | 12 | 103230663 | 103352188 | 36 | 6.65E-06 | 0.004799086 |
| 6 | ENSG00000126733 | DACH2 | Dachshund homolog 2 (Drosophila) | X | 85403462 | 86087607 | 36 | 7.24E-06 | 0.005048563 |
| 7 | ENSG00000240045 | RP11-451G4.2 | | 3 | 155008018 | 155011564 | 48 | 1.02E-05 | 0.006800863 |
| 8 | ENSG00000227560 | RP11-139K1.2 | | 10 | 114747235 | 114747624 | 46 | 1.12E-05 | 0.007322341 |
| 9 | ENSG00000170373 | CST1 | Cystatin SN | 20 | 23728190 | 23731905 | 29 | 1.67E-05 | 0.009967044 |
| 10 | ENSG00000153294 | GPR115 | G protein-coupled receptor 115 | 6 | 47653600 | 47689757 | 40 | 2.42E-05 | 0.013316272 |
| 11 | ENSG00000254349 | RP11-758M4.1 | | 8 | 75512010 | 75735548 | 29 | 3.75E-05 | 0.020150228 |
| 12 | ENSG00000134443 | GRP | Gastrin-releasing peptide | 18 | 56887400 | 56898006 | 25 | 4.08E-05 | 0.020883677 |
| 13 | ENSG00000171560 | FGA | Fibrinogen alpha chain | 4 | 155504278 | 155511918 | 225 | 4.28E-05 | 0.021347954 |
| 14 | ENSG00000100665 | SERPINA4 | Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, | 14 | 95027428 | 95036250 | 63 | 4.48E-05 | 0.021798893 |
| 15 | ENSG00000230327 | AC009234.1 | | 2 | 50815828 | 50816553 | 39 | 4.60E-05 | 0.021798893 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | ENSG00000170426 | SDR9C7 | Short chain dehydrogenase/reductase family 9C, member 7 | 12 | 57316938 | 57328189 | 25 | 5.60E-05 | 0.025738821 |
| 17 | ENSG00000154975 | CA10 | Carbonic anhydrase X | 17 | 49707674 | 50237377 | 36 | 6.09E-05 | 0.02656543 |
| 18 | ENSG00000237567 | RP3-359N14.2 | | 6 | 106060542 | 106080071 | 24 | 7.39E-05 | 0.030073102 |
| 19 | ENSG00000095627 | TDRD1 | Tudor domain containing 1 | 10 | 115939029 | 115992063 | 23 | 7.52E-05 | 0.030073102 |
| 20 | ENSG00000224099 | AC064834.1 | | 2 | 196313256 | 196343649 | 31 | 8.66E-05 | 0.033874159 |
| 21 | ENSG00000146039 | SLC17A4 | Solute carrier family 17 (sodium phosphate), member 4 | 6 | 25754927 | 25781419 | 27 | 8.89E-05 | 0.034471943 |
| 22 | ENSG00000261045 | RP11-673P17.4 | | 16 | 27169775 | 27187908 | 22 | 0.000107141 | 0.038504 |
| 23 | ENSG00000243236 | RP1-214M20.2 | | 6 | 52804524 | 52822319 | 18 | 0.000117679 | 0.041050234 |
| 24 | ENSG00000156925 | ZIC3 | ZIC family member 3 | X | 136648301 | 136659850 | 53 | 0.000129321 | 0.044011046 |
| 25 | ENSG00000128564 | VGF | VGF nerve growth factor inducible | 7 | 100805790 | 100808874 | 19 | 0.000145369 | 0.047914417 |
| 26 | ENSG00000187416 | LHFPL3 | Lipoma HMGIC fusion partner-like 3 | 7 | 103969104 | 104549001 | 21 | 0.000150205 | 0.049121823 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 9.** Differentially expressed genes (FDR < 0.05) down-regulated by soy isoflavone supplementation of the 186 differentially expressed genes from normal tissue obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ENSG00000136750 | GAD2 | Glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa) | 10 | 26505236 | 26593487 | 83 | 2.92E-14 | 1.33E-09 |
| 2 | ENSG00000234931 | MARK2P15 | MAP/microtubule affinity-regulating kinase 2 pseudogene 15 | 10 | 85071384 | 85074231 | 61 | 1.53E-13 | 3.49E-09 |
| 3 | ENSG00000244734 | HBB | Hemoglobin, beta | 11 | 5246694 | 5250625 | 33 | 2.31E-13 | 3.52E-09 |
| 4 | ENSG00000205271 | RP11-152F13.4 | | 15 | 83128128 | 83145983 | 93 | 3.27E-13 | 3.73E-09 |
| 5 | ENSG00000257846 | RP11-597A11.3 | | 10 | 27035522 | 27150016 | 34 | 3.34E-12 | 3.05E-08 |
| 6 | ENSG00000235992 | GRAMD4P2 | GRAM domain containing 4 pseudogene 2 | 22 | 16227138 | 16228807 | 25 | 1.17E-11 | 8.93E-08 |
| 7 | ENSG00000260125 | RP11-31E22.1 | | 15 | 86838880 | 86860404 | 44 | 5.47E-11 | 3.56E-07 |
| 8 | ENSG00000168824 | NSG1 | Neuron-specific protein family member 1 | 4 | 4349867 | 4420785 | 21 | 8.87E-11 | 5.06E-07 |
| 9 | ENSG00000244342 | LINC00698 | Long intergenic non-protein coding RNA 698 | 10 | 27399383 | 27444195 | 40 | 1.87E-10 | 9.47E-07 |
| 10 | ENSG00000225960 | RP11-360A18.1 | | 9 | 122735372 | 122736600 | 53 | 3.31E-10 | 1.44E-06 |
| 11 | ENSG00000259278 | RP11-62C7.2 | | 15 | 39311434 | 39317119 | 25 | 3.49E-10 | 1.44E-06 |
| 12 | ENSG00000259379 | RP11-925D8.5 | | 15 | 58444805 | 58446606 | 88 | 5.08E-10 | 1.93E-06 |
| 13 | ENSG00000254861 | RP11-945A11.2 | | 11 | 23782876 | 23826144 | 18 | 2.44E-09 | 7.41E-06 |
| 14 | ENSG00000112280 | COL9A1 | Collagen, type IX, alpha 1 | 6 | 70924764 | 71012786 | 16 | 2.66E-09 | 7.58E-06 |
| 15 | ENSG00000166006 | KCNC2 | Potassium voltage-gated channel, Shaw-related subfamily, member | 12 | 75433857 | 75603648 | 15 | 3.42E-09 | 9.18E-06 |

2

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | ENSG00000258590 | NBEAP1 | Neurobeachin pseudogene 1 | 15 | 20862967 | 20893737 | 15 | 3.87E-09 | 9.80E-06 |
| 17 | ENSG00000024526 | DEPDC1 | DEP domain containing 1 | 1 | 68939835 | 68962904 | 15 | 7.13E-09 | 1.71E-05 |
| 18 | ENSG00000257751 | RP11-536C10.21 | | 10 | 22045466 | 22292698 | 66 | 1.10E-08 | 2.39E-05 |
| 19 | ENSG00000223609 | HBD | Hemoglobin, delta | 11 | 5253908 | 5256600 | 151 | 1.38E-08 | 2.86E-05 |
| 20 | ENSG00000259472 | RP13-996F3.3 | | 15 | 83141718 | 83182928 | 147 | 1.84E-08 | 3.64E-05 |
| 21 | ENSG00000264063 | MIR3687 | MicroRNA 3687 | 21 | 9826203 | 9826263 | 14 | 2.04E-08 | 3.87E-05 |
| 22 | ENSG00000002726 | ABP1 | Amiloride binding protein 1 (amine oxidase (copper containing)) | 7 | 150521715 | 150558592 | 13 | 2.81E-08 | 5.13E-05 |
| 23 | ENSG00000213727 | LA16c-60G3.7 | | 22 | 16404698 | 16405874 | 22 | 2.98E-08 | 5.23E-05 |
| 24 | ENSG00000170893 | TRH | Thyrotropin-releasing hormone | 3 | 129693148 | 129696781 | 30 | 4.03E-08 | 6.81E-05 |
| 25 | ENSG00000258027 | RP11-597A11.1 | | 14 | 20078033 | 20146082 | 22 | 4.68E-08 | 7.63E-05 |
| 26 | ENSG00000199916 | RMRP | RNA component of mitochondrial RNA processing endoribonuclease | 9 | 35657751 | 35658014 | 13 | 5.10E-08 | 8.02E-05 |
| 27 | ENSG00000223542 | RP1-283K11.3 | | 6 | 133756215 | 133760602 | 21 | 5.41E-08 | 8.23E-05 |
| 28 | ENSG00000096006 | CRISP3 | Cysteine-rich secretory protein 3 | 6 | 49695097 | 49712150 | 12 | 6.41E-08 | 9.40E-05 |
| 29 | ENSG00000257395 | RP11-597A11.2 | | 14 | 20149579 | 20152898 | 24 | 6.60E-08 | 9.40E-05 |
| 30 | ENSG00000258363 | RP11-146E13.6 | | 14 | 19959517 | 19961162 | 12 | 7.14E-08 | 9.78E-05 |
| 31 | ENSG00000121742 | GJB6 | Gap junction protein, beta 6, 30kDa | 9 | 20796110 | 20806534 | 13 | 7.29E-08 | 9.78E-05 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 32 | ENSG00000184115 | RP13-1039J1.2 | | 2 | 111143988 | 111192743 | 23 | 7.89E-08 | 0.00010277 |
| 33 | ENSG00000237754 | RP11-521C10.1 | | 7 | 67706624 | 67707254 | 17 | 1.11E-07 | 0.000140766 |
| 34 | ENSG00000225255 | LA16c-83F12.6 | | 22 | 16199666 | 16231333 | 11 | 1.45E-07 | 0.000169403 |
| 35 | ENSG00000265971 | RP11-269G24.6 | | 17 | 61532354 | 61533178 | 15 | 1.49E-07 | 0.000169403 |
| 36 | ENSG00000259761 | RP11-138H10.2 | | 15 | 87585285 | 87590392 | 119 | 1.65E-07 | 0.000183268 |
| 37 | ENSG00000257731 | RP11-536C10.11 | | 14 | 19421521 | 19422698 | 14 | 1.82E-07 | 0.00019736 |
| 38 | ENSG00000254851 | RP11-109L13.1 | | 11 | 117006244 | 117009298 | 16 | 1.90E-07 | 0.000201776 |
| 39 | ENSG00000157884 | CIB4 | Calcium and integrin binding family member 4 | 2 | 26804070 | 26864236 | 12 | 2.15E-07 | 0.000222688 |
| 40 | ENSG00000217889 | RP11-66N11.5 | | X | 152869952 | 152870803 | 25 | 2.57E-07 | 0.000260106 |
| 41 | ENSG00000100121 | GGTLC2 | Gamma-glutamyltransferase light chain 2 | 22 | 22988780 | 22990368 | 12 | 2.90E-07 | 0.000287678 |
| 42 | ENSG00000259671 | RP11-925D8.6 | | 15 | 58447159 | 58448260 | 46 | 4.25E-07 | 0.000412623 |
| 43 | ENSG00000257357 | RP11-244H18.3 | | 14 | 20105463 | 20109540 | 23 | 5.50E-07 | 0.000522509 |
| 44 | ENSG00000232783 | AC073135.3 | | 3 | 197836983 | 197838749 | 37 | 6.65E-07 | 0.000594735 |
| 45 | ENSG00000234665 | RP11-262H14.3 | | 9 | 66513488 | 66553911 | 19 | 6.65E-07 | 0.000594735 |
| 46 | ENSG00000225383 | SFTA1P | Surfactant associated 1, pseudogene | 10 | 10826400 | 10836943 | 23 | 7.16E-07 | 0.000627728 |
| 47 | ENSG00000224371 | RP11-235G24.1 | | 9 | 161293920 | 161339681 | 18 | 8.21E-07 | 0.000701696 |
| 48 | ENSG00000224758 | RP13-137A17.5 | | 10 | 134774844 | 134775741 | 17 | 8.31E-07 | 0.000701696 |
| 49 | ENSG00000203697 | CAPN8 | Calpain 8 | 1 | 223711349 | 223853436 | 9 | 8.99E-07 | 0.000745586 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 50 | ENSG00000257504 | RP11-536C10.7 | | 9 | 19406795 | 19410111 | 10 | 1.02E-06 | 0.000818554 |
| 51 | ENSG00000188536 | HBA2 | Hemoglobin, alpha 2 | 16 | 222846 | 223709 | 9 | 1.02E-06 | 0.000818554 |
| 52 | ENSG00000236045 | RP3-467K16.7 | | 9 | 15660662 | 15661960 | 10 | 1.59E-06 | 0.001178183 |
| 53 | ENSG00000238245 | MYO5BP2 | Myosin VB pseudogene 2 | 9 | 66513032 | 66514308 | 29 | 1.63E-06 | 0.001178183 |
| 54 | ENSG00000228897 | CTD-2021A8.2 | | 7 | 51454060 | 51454798 | 28 | 1.63E-06 | 0.001178183 |
| 55 | ENSG00000257608 | CTD-2311B13.6 | | 9 | 19614216 | 19615884 | 9 | 1.66E-06 | 0.001178363 |
| 56 | ENSG00000267242 | AC069278.4 | | 19 | 44978645 | 44979403 | 9 | 1.73E-06 | 0.001193033 |
| 57 | ENSG00000183091 | NEB | Nebulin | 9 | 152341850 | 152591001 | 9 | 1.84E-06 | 0.001232515 |
| 58 | ENSG00000222375 | RN7SKP127 | RNA, 7SK small nuclear pseudogene 127 | 16 | 29742372 | 29742725 | 9 | 1.84E-06 | 0.001232515 |
| 59 | ENSG00000234567 | RP1-283K11.2 | | 6 | 133773995 | 133777743 | 12 | 1.87E-06 | 0.001232515 |
| 60 | ENSG00000202198 | RN7SK | RNA, 7SK small nuclear | 9 | 52860418 | 52860748 | 9 | 2.18E-06 | 0.001422985 |
| 61 | ENSG00000233845 | AC093732.1 | | 2 | 47262418 | 47267663 | 16 | 2.26E-06 | 0.001449668 |
| 62 | ENSG00000230223 | ATXN8OS | ATXN8 opposite strand (non-protein coding) | 13 | 70681345 | 70705678 | 13 | 2.69E-06 | 0.001704723 |
| 63 | ENSG00000206172 | HBA1 | Hemoglobin, alpha 1 | 16 | 226679 | 227521 | 9 | 2.93E-06 | 0.001829279 |
| 64 | ENSG00000237674 | GSTA7P | Glutathione S-transferase alpha 7, pseudogene | 6 | 52604388 | 52609454 | 16 | 3.12E-06 | 0.001923142 |
| 65 | ENSG00000163618 | CADPS | Ca++-dependent secretion activator | 3 | 62384022 | 62861054 | 8 | 3.27E-06 | 0.001963106 |
| 66 | ENSG00000226958 | RNA28S5 | RNA, 28S ribosomal 5 | X | 108297361 | 108297792 | 8 | 3.41E-06 | 0.002012606 |
| 67 | ENSG00000260581 | CTB-113P19.4 | | 5 | 151031836 | 151035010 | 9 | 3.44E-06 | 0.002012606 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 68 | ENSG00000253998 | IGKV2-29 | Immunoglobulin kappa variable 2-29 | 2 | 89533655 | 89534393 | 40 | 3.89E-06 | 0.002192593 |
| 69 | ENSG00000225649 | AC064875.2 | | 2 | 13106910 | 13147138 | 8 | 4.36E-06 | 0.002426373 |
| 70 | ENSG00000150361 | KLHL1 | Kelch-like family member 1 | 13 | 70274726 | 70682591 | 9 | 4.84E-06 | 0.002632142 |
| 71 | ENSG00000261760 | RP11-1223D19.1 | | 2 | 110988683 | 111043433 | 82 | 5.22E-06 | 0.002802477 |
| 72 | ENSG00000233306 | TRGV2 | T cell receptor gamma variable 2 | 7 | 38402465 | 38403119 | 10 | 5.31E-06 | 0.00281554 |
| 73 | ENSG00000233408 | LA16c-23H5.4 | | 22 | 16417269 | 16420386 | 12 | 5.47E-06 | 0.002868297 |
| 74 | ENSG00000170356 | OR2A20P | Olfactory receptor, family 2, subfamily A, member 20 pseudogene | 7 | 143947138 | 143950050 | 11 | 6.71E-06 | 0.003474592 |
| 75 | ENSG00000162344 | FGF19 | Fibroblast growth factor 19 | 11 | 69513000 | 69519410 | 14 | 8.28E-06 | 0.004103311 |
| 76 | ENSG00000238881 | SCARNA2 | Small Cajal body-specific RNA 2 | 1 | 109642815 | 109643234 | 8 | 9.34E-06 | 0.004580364 |
| 77 | ENSG00000258730 | ITPK1-AS1 | ITPK1 antisense RNA 1 | 14 | 93533797 | 93538497 | 17 | 9.87E-06 | 0.004788171 |
| 78 | ENSG00000211699 | TRGV3 | T cell receptor gamma variable 3 | 7 | 38398113 | 38398763 | 10 | 1.01E-05 | 0.004855999 |
| 79 | ENSG00000260555 | RP11-728K20.2 | | 7 | 149697841 | 149702213 | 9 | 1.12E-05 | 0.005311565 |
| 80 | ENSG00000251060 | U66061.31 | | 7 | 142413074 | 142426272 | 10 | 1.33E-05 | 0.006074933 |
| 81 | ENSG00000225210 | AL589743.1 | | 14 | 19650018 | 19718563 | 7 | 1.40E-05 | 0.006338535 |
| 82 | ENSG00000211688 | TRGJP2 | T cell receptor gamma joining P2 | 7 | 38295938 | 38295997 | 10 | 1.46E-05 | 0.006544322 |
| 83 | ENSG00000244067 | GSTA2 | Glutathione S-transferase alpha 2 | 6 | 52614897 | 52628367 | 7 | 1.53E-05 | 0.00673148 |
| 84 | ENSG00000249170 | RP11-1J11.1 | | 4 | 72165975 | 72166952 | 12 | 1.66E-05 | 0.007148469 |
| 85 | ENSG00000232079 | AL035610.1 | | 21 | 29420733 | 29509930 | 13 | 1.77E-05 | 0.007533531 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 86 | ENSG00000250984 | CTD-2011G17.1 | | 5 | 29796045 | 29796375 | 15 | 1.90E-05 | 0.008028562 |
| 87 | ENSG00000258265 | CTD-2311B13.2 | | 14 | 19529958 | 19530898 | 69 | 2.07E-05 | 0.008578552 |
| 88 | ENSG00000225258 | AC009478.1 | | 2 | 181436439 | 181557181 | 10 | 2.19E-05 | 0.008898036 |
| 89 | ENSG00000180532 | ZSCAN4 | Zinc finger and SCAN domain containing 4 | 19 | 58180303 | 58190520 | 7 | 2.43E-05 | 0.00978666 |
| 90 | ENSG00000138100 | TRIM54 | Tripartite motif containing 54 | 2 | 27505260 | 27530307 | 7 | 2.59E-05 | 0.010378663 |
| 91 | ENSG00000231565 | NEK2P2 | NEK2 pseudogene 2 | 22 | 16364867 | 16366204 | 7 | 2.66E-05 | 0.010533754 |
| 92 | ENSG00000227195 | RP3-410C9.1 | | 20 | 26167556 | 26232162 | 6 | 3.07E-05 | 0.012076346 |
| 93 | ENSG00000164093 | PITX2 | Paired-like homeodomain 2 | 4 | 111538579 | 111563279 | 8 | 3.11E-05 | 0.012115254 |
| 94 | ENSG00000169856 | ONECUT1 | One cut homeobox 1 | 15 | 53049186 | 53083273 | 65 | 3.38E-05 | 0.012962553 |
| 95 | ENSG00000214976 | VDAC2P1 | Voltage-dependent anion channel 2 pseudogene 1 | 21 | 17466735 | 17467692 | 62 | 3.38E-05 | 0.012962553 |
| 96 | ENSG00000117154 | IGSF21 | Immunoglobulin superfamily, member 21 | 1 | 18434240 | 18704977 | 6 | 3.77E-05 | 0.014250253 |
| 97 | ENSG00000166748 | AGBL1 | ATP/GTP binding protein-like 1 | 15 | 86685227 | 87572283 | 8 | 3.78E-05 | 0.014250253 |
| 98 | ENSG00000239736 | CEACAMP3 | Carcinoembryonic antigen-related cell adhesion molecule pseudogene 3 | 19 | 42106090 | 42112339 | 20 | 3.82E-05 | 0.014262985 |
| 99 | ENSG00000204038 | AL359195.1 | | 10 | 82009466 | 82013395 | 27 | 3.99E-05 | 0.014672388 |
| 100 | ENSG00000206177 | HBM | Hemoglobin, mu | 16 | 203891 | 216767 | 27 | 3.99E-05 | 0.014672388 |
| 101 | ENSG00000267614 | AC138472.6 | | 19 | 45040251 | 45040599 | 11 | 4.45E-05 | 0.01622454 |
| 102 | ENSG00000259425 | RP11-566K19.5 | | 15 | 23096869 | 23105332 | 7 | 4.99E-05 | 0.017634483 |
| 103 | ENSG00000228477 | RP3-342P20.2 | | 1 | 40428352 | 40429076 | 9 | 5.34E-05 | 0.018747218 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 104 | ENSG00000254127 | IGLCOR22-1 | Immunoglobulin lambda constant/OR22-1 | 22 | 32595906 | 32596221 | 14 | 5.40E-05 | 0.018800627 |
| 105 | ENSG00000169427 | KCNK9 | Potassium channel, subfamily K, member 9 | 8 | 140613081 | 140715299 | 58 | 5.63E-05 | 0.019274052 |
| 106 | ENSG00000261426 | AC144833.1 | | 15 | 27607456 | 27611544 | 58 | 5.63E-05 | 0.019274052 |
| 107 | ENSG00000236714 | AC005592.1 | | 5 | 142125165 | 142140563 | 6 | 5.99E-05 | 0.020239635 |
| 108 | ENSG00000166816 | LDHD | Lactate dehydrogenase D | 16 | 75145758 | 75150669 | 6 | 6.06E-05 | 0.020334552 |
| 109 | ENSG00000178934 | LGALS7B | Lectin, galactoside-binding, soluble, 7B | 19 | 39279851 | 39282389 | 7 | 6.35E-05 | 0.021030946 |
| 110 | ENSG00000148826 | NKX6-2 | NK6 homeobox 2 | 10 | 134598297 | 134599556 | 20 | 6.37E-05 | 0.021030946 |
| 111 | ENSG00000244280 | ECEL1P2 | Endothelin converting enzyme-like 1, pseudogene 2 | 2 | 233250460 | 233252167 | 7 | 6.41E-05 | 0.021030946 |
| 112 | ENSG00000259344 | RP11-566K19.6 | | 15 | 23095170 | 23115256 | 6 | 6.57E-05 | 0.021387892 |
| 113 | ENSG00000253988 | RP11-489O18.1 | | 8 | 139075511 | 139085483 | 8 | 6.99E-05 | 0.022605697 |
| 114 | ENSG00000255193 | RP11-945A11.1 | | 11 | 23752134 | 23818516 | 7 | 7.06E-05 | 0.022683372 |
| 115 | ENSG00000256642 | LINC00273 | Long intergenic non-protein coding RNA 273 | 16 | 33961052 | 33962503 | 6 | 7.40E-05 | 0.023114368 |
| 116 | ENSG00000173809 | TDRD12 | Tudor domain containing 12 | 19 | 33210659 | 33320483 | 6 | 7.63E-05 | 0.023665842 |
| 117 | ENSG00000248408 | RP11-452C8.1 | | 4 | 80584915 | 80617991 | 6 | 7.72E-05 | 0.023667538 |
| 118 | ENSG00000109158 | GABRA4 | Gamma-aminobutyric aicd (GABA) A receptor, alpha 4 | 4 | 46920917 | 46996424 | 13 | 7.79E-05 | 0.023667538 |
| 119 | ENSG00000261467 | RP11-731K22.1 | | 7 | 73400322 | 73403097 | 13 | 7.79E-05 | 0.023667538 |
| 120 | ENSG00000234232 | RP11-353N4.5 | | 1 | 149691432 | 149698605 | 24 | 9.34E-05 | 0.028201554 |
| 121 | ENSG00000152969 | JAKMIP1 | Janus kinase and microtubule interacting protein 1 | 4 | 6027926 | 6202318 | 6 | 9.95E-05 | 0.029754851 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 122 | ENSG00000257898 | | | | | | 6 | 9.98E-05 | 0.029754851 |
| 123 | ENSG00000142619 | PADI3 | Peptidyl arginine deiminase, type III | 1 | 17575593 | 17610728 | 6 | 0.000104146 | 0.030836563 |
| 124 | ENSG00000264462 | MIR3648 | MicroRNA 3648 | 21 | 9825832 | 9826011 | 6 | 0.000105754 | 0.031110755 |
| 125 | ENSG00000260284 | TPSP2 | Tryptase pseudogene 2 | 16 | 1336352 | 1338838 | 6 | 0.000106571 | 0.031150138 |
| 126 | ENSG00000236138 | RP11-413E6.7 | | 3 | 75718082 | 75719336 | 7 | 0.000111474 | 0.032375703 |
| 127 | ENSG00000230666 | CEACAM22P | Carcinoembryonic antigen-related cell adhesion molecule 2, pseudogene | 19 | 45051045 | 45124119 | 6 | 0.000115579 | 0.03314582 |
| 128 | ENSG00000257884 | RP11-587A11.4 | | 14 | 20145996 | 20148263 | 6 | 0.000122949 | 0.034617353 |
| 129 | ENSG00000235994 | RP3-470B24.5 | | 6 | 168376604 | 168380456 | 11 | 0.000122988 | 0.034617353 |
| 130 | ENSG00000205076 | LGALS7 | Lectin, galactoside-binding, souble, 7 | 19 | 39261611 | 39264132 | 11 | 0.000122988 | 0.034617353 |
| 131 | ENSG00000252139 | SCARNA18 | Small Cajal body-specific RNA 18 | 18 | 47340731 | 47340813 | 15 | 0.00012697 | 0.035518933 |
| 132 | ENSG00000180383 | DEFB124 | Defensin, beta 124 | 20 | 30053309 | 30064560 | 7 | 0.000128563 | 0.035745202 |
| 133 | ENSG00000170509 | HSD17B13 | Hydroxysteroid (17-beta) dehydrogenase 13 | 4 | 88224941 | 88244058 | 6 | 0.000132806 | 0.036480179 |
| 134 | ENSG00000132204 | LINC00470 | Long intergenic non-protein coding RNA 470 | 18 | 1254384 | 1408345 | 7 | 0.000137231 | 0.037469935 |
| 135 | ENSG00000237686 | RP5-1120P11.1 | | 6 | 43963460 | 44042389 | 6 | 0.000143964 | 0.039074183 |
| 136 | ENSG00000253326 | RP11-261C10.7 | | 1 | 243218163 | 243219696 | 23 | 0.000145771 | 0.039330509 |
| 137 | ENSG00000259600 | RP11-925D8.3 | | 15 | 58443232 | 58444712 | 8 | 0.000152739 | 0.04072871 |
| 138 | ENSG00000206195 | AP000525.9 | | 22 | 16147979 | 16193004 | 6 | 0.000154801 | 0.041038475 |
| 139 | ENSG00000266998 | RP11-936I5.1 | | 17 | 75369900 | 75373318 | 9 | 0.00016348 | 0.042841205 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 140 | ENSG00000221673 | U3 | Small nucleolar RNA U3 | 1 | 220136028 | 220136206 | 49 | 0.000165955 | 0.042995614 |
| 141 | ENSG00000240881 | RP11-713P14.1 | | 11 | 23541868 | 23542748 | 49 | 0.000165955 | 0.042995614 |
| 142 | ENSG00000170423 | KRT78 | Keratin 78 | 12 | 53231588 | 53242876 | 10 | 0.000168481 | 0.043159648 |
| 143 | ENSG00000166268 | MYRFL | Myelin regulatory factor-like | 12 | 70219084 | 70352877 | 6 | 0.000170898 | 0.043533352 |
| 144 | ENSG00000259787 | CTB-85P21.2 | | 5 | 143861871 | 143865249 | 10 | 0.000172198 | 0.043533352 |
| 145 | ENSG00000263015 | RP5-1029F21.3 | | 17 | 406411 | 414587 | 11 | 0.000172804 | 0.043533352 |
| 146 | ENSG00000188000 | OR7D2 | Olfactory receptor, family 7, subfamily D, member 2 | 19 | 9296279 | 9299493 | 7 | 0.000182463 | 0.045464119 |
| 147 | ENSG00000223764 | RP11-54O7.3 | | 1 | 852250 | 855072 | 5 | 0.000195371 | 0.048154249 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 10.** Differentially expressed genes (FDR < 0.05) up-regulated by soy isoflavone supplementation of the 186 differentially expressed genes from normal tissue obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ENSG00000206073 | SERPINB4 | Serpin peptidase inhibitor, clade B (ovalbumin), member 4 | 18 | 61304493 | 61311532 | 69 | 7.13E-10 | 2.50E-06 |
| 2 | ENSG00000164266 | SPINK1 | Serine peptidase inhibitor, Kazal type 1 | 5 | 147204131 | 147211349 | 63 | 1.01E-09 | 3.28E-06 |
| 3 | ENSG00000226278 | PSPHP1 | Phosphoserine phosphatase pseudogene 1 | 7 | 55832490 | 55840981 | 100 | 8.43E-09 | 1.92E-05 |
| 4 | ENSG00000170369 | CST2 | Cystatin SA | 20 | 23804406 | 23807368 | 27 | 1.15E-07 | 0.000141837 |
| 5 | ENSG00000057149 | SERPINB3 | Serpin peptidase inhibitor, clade B (ovalbumin), member 3 | 18 | 61322431 | 61329197 | 32 | 1.33E-07 | 0.000158996 |
| 6 | ENSG00000264209 | AC104448.1 | | 3 | 48529745 | 48529838 | 266 | 6.30E-07 | 0.000586159 |
| 7 | ENSG00000171564 | FGB | Fibrinogen beta chain | 9 | 155484108 | 155492236 | 67 | 1.11E-06 | 0.000869858 |
| 8 | ENSG00000247627 | MTND4P12 | MT-ND4 pseudogene 12 | 5 | 134262350 | 134263726 | 19 | 1.13E-06 | 0.000869858 |
| 9 | ENSG00000267056 | AC005336.4 | | 19 | 16021393 | 16022634 | 18 | 1.49E-06 | 0.001134641 |
| 10 | ENSG00000156096 | UGT2B4 | UDP glucuronosyltransferase 2 family, polypeptide B4 | 4 | 70345883 | 70391732 | 26 | 1.68E-06 | 0.001178363 |
| 11 | ENSG00000175445 | LPL | Lipoprotein lipase | 9 | 19759228 | 19824769 | 16 | 3.25E-06 | 0.001963106 |
| 12 | ENSG00000163735 | CXCL5 | Chemokine (C-X-C motif) ligand 5 | 4 | 74861359 | 74864496 | 16 | 3.54E-06 | 0.002038721 |
| 13 | ENSG00000182816 | KRTAP13-2 | Keratin associated protein 13-2 | 21 | 31743709 | 31744557 | 40 | 3.58E-06 | 0.002038721 |
| 14 | ENSG00000163209 | SPRR3 | Small proline-rich protein 3 | 1 | 152974223 | 152976332 | 29 | 4.85E-06 | 0.002632142 |
| 15 | ENSG00000000005 | TNMD | Tenomodulin | X | 99839799 | 99854882 | 18 | 7.34E-06 | 0.003746265 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | ENSG00000184937 | WT1 | Wilms tumor 1 | 11 | 32409321 | 32457176 | 15 | 7.39E-06 | 0.003746265 |
| 17 | ENSG00000251165 | RP11-215A19.1 | | 4 | 187207248 | 187422151 | 49 | 7.58E-06 | 0.003795706 |
| 18 | ENSG00000134339 | SAA2 | Serum amyloid A2 | 11 | 18260770 | 18270190 | 13 | 1.14E-05 | 0.005357755 |
| 19 | ENSG00000172238 | ATOH1 | Atonal homolog 1 (Drosophila) | 4 | 94750042 | 94751221 | 22 | 1.15E-05 | 0.005357755 |
| 20 | ENSG00000227063 | RPL41P1 | Ribosomal protein L41 pseudogene 1 | 20 | 21735866 | 21736171 | 17 | 1.20E-05 | 0.005515008 |
| 21 | ENSG00000243063 | IGKV3-7 | Immunoglobulin kappa variable 3-7 (non-functional) | 2 | 89277987 | 89278600 | 161 | 1.55E-05 | 0.00673148 |
| 22 | ENSG00000143556 | S100A7 | S100 calcium binding protein A7 | 1 | 153430220 | 153433177 | 155 | 1.55E-05 | 0.00673148 |
| 23 | ENSG00000211974 | IGHV2-70 | Immunoglobulin heavy variable 2-70 | 14 | 107178820 | 107179338 | 13 | 1.93E-05 | 0.008055596 |
| 24 | ENSG00000198535 | C2CD4A | C2 calcium-dependent domain containing 4A | 15 | 62359176 | 62363116 | 12 | 2.17E-05 | 0.008898036 |
| 25 | ENSG00000099290 | FAM21A | Family with sequence similarity 21, member A | 10 | 51827648 | 51893269 | 11 | 4.90E-05 | 0.017634483 |
| 26 | ENSG00000231083 | AC011747.6 | | 2 | 8741022 | 8763072 | 36 | 4.93E-05 | 0.017634483 |
| 27 | ENSG00000196427 | NBPF4 | Neuroblastoma breakpoint family, member 4 | 1 | 108765087 | 108786689 | 130 | 4.98E-05 | 0.017634483 |
| 28 | ENSG00000104267 | CA2 | Carbonic anhydrase II | 8 | 86376081 | 86393722 | 10 | 5.66E-05 | 0.019274052 |
| 29 | ENSG00000198178 | CLEC4C | C-type lectin domain family 4, member C | 12 | 7882011 | 7904201 | 116 | 7.22E-05 | 0.023032885 |
| 30 | ENSG00000233760 | AC004947.2 | | 7 | 26591441 | 26596819 | 11 | 7.32E-05 | 0.023114368 |
| 31 | ENSG00000229403 | RP4-718N17.2 | | 7 | 51681947 | 51698566 | 15 | 7.36E-05 | 0.023114368 |
| 32 | ENSG00000069206 | ADAM7 | ADAM metallopeptidase domain 7 | 8 | 24298443 | 24384483 | 13 | 0.00011541 | 0.03314582 |
| 33 | ENSG00000104760 | FGL1 | Fibrinogen-like 1 | 8 | 17721889 | 17767874 | 12 | 0.00013199 | 0.036475647 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 34 | ENSG00000095917 | TPSD1 | Tryptase delta 1 | 16 | 1306060 | 1308532 | 10 | 0.000149058 | 0.039980789 |
| 35 | ENSG00000166396 | SERPINB7 | Serpin peptidase inhibitor, clade B (ovalbumin), member 7 | 18 | 61420169 | 61472604 | 10 | 0.00015935 | 0.042000137 |
| 36 | ENSG00000119913 | TECTB | Tectorin beta | 10 | 114043493 | 114064793 | 11 | 0.000168324 | 0.043159648 |
| 37 | ENSG00000121075 | TBX4 | T-box 4 | 17 | 59529765 | 59562471 | 9 | 0.000180519 | 0.045226933 |
| 38 | ENSG00000164756 | SLC30A8 | Solute carrier family 30 (zinc transporter), member 8 | 8 | 117962512 | 118188953 | 17 | 0.000185505 | 0.045970975 |
| 39 | ENSG00000244921 | CTB-36O1.7 | | 5 | 134258993 | 134259739 | 9 | 0.000201827 | 0.049478048 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 11.** Differentially expressed genes (FDR <0.05) down-regulated by soy isoflavone supplementation of the 166 differentially expressed genes from only normal tissue obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ENSG00000136750 | GAD2 | Glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa) | 10 | 26505236 | 26593487 | 83 | 2.92E-14 | 1.33E-09 |
| 2 | ENSG00000244734 | HBB | Hemoglobin, beta | 11 | 5246694 | 5250625 | 33 | 2.31E-13 | 3.52E-09 |
| 3 | ENSG00000205271 | RP11-152F13.4 | | 15 | 83128128 | 83145983 | 93 | 3.27E-13 | 3.73E-09 |
| 4 | ENSG00000257846 | RP11-597A11.3 | | 14 | 20136988 | 20138162 | 34 | 3.34E-12 | 3.05E-08 |
| 5 | ENSG00000235992 | GRAMD4P2 | GRAM domain containing 4 pseudogene 2 | 22 | 16227138 | 16228807 | 25 | 1.17E-11 | 8.93E-08 |
| 6 | ENSG00000168824 | NSG1 | Neuron-specific protein family member 1 | 4 | 4349867 | 4420785 | 21 | 8.87E-11 | 5.06E-07 |
| 7 | ENSG00000244342 | LINC00698 | Long intergenic non-protein coding RNA 698 | 3 | 62936105 | 63110738 | 40 | 1.87E-10 | 9.47E-07 |
| 8 | ENSG00000225960 | RP11-360A18.1 | | 9 | 122735372 | 122736600 | 53 | 3.31E-10 | 1.44E-06 |
| 9 | ENSG00000259278 | RP11-62C7.2 | | 15 | 39311434 | 39317119 | 25 | 3.49E-10 | 1.44E-06 |
| 10 | ENSG00000259379 | RP11-925D8.5 | | 15 | 58444805 | 58446606 | 88 | 5.08E-10 | 1.93E-06 |
| 11 | ENSG00000254861 | RP11-945A11.2 | | 11 | 23782876 | 23826144 | 18 | 2.44E-09 | 7.41E-06 |
| 12 | ENSG00000112280 | COL9A1 | Collagen, type IX, alpha 1 | 6 | 70924764 | 71012786 | 16 | 2.66E-09 | 7.58E-06 |
| 13 | ENSG00000166006 | KCNC2 | Potassium voltage-gated channel, Shaw-realted | 12 | 75433857 | 75603648 | 15 | 3.42E-09 | 9.18E-06 |
| 14 | ENSG00000258590 | NBEAP1 | Neurobeachin pseudogene 1 | 15 | 20862967 | 20893737 | 15 | 3.87E-09 | 9.80E-06 |
| 15 | ENSG00000024526 | DEPDC1 | DEP domain containing 1 | 1 | 68939835 | 68962904 | 15 | 7.13E-09 | 1.71E-05 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | ENSG00000257751 | RP11-536C10.21 | | 14 | 20136701 | 20137215 | 66 | 1.10E-08 | 2.39E-05 |
| 17 | ENSG00000223609 | HBD | Hemoglobin, delta | 11 | 5253908 | 5256600 | 151 | 1.38E-08 | 2.86E-05 |
| 18 | ENSG00000259472 | RP13-996F3.3 | | 15 | 83141718 | 83182928 | 147 | 1.84E-08 | 3.64E-05 |
| 19 | ENSG00000264063 | MIR3687 | MicroRNA 3687 | 21 | 9826203 | 9826263 | 14 | 2.04E-08 | 3.87E-05 |
| 20 | ENSG00000002726 | ABP1 | Amiloride binding protein 1 (amine oxidase | 7 | 150521715 | 150558592 | 13 | 2.81E-08 | 5.13E-05 |
| 21 | ENSG00000213727 | LA16c-60G3.7 | | 22 | 16404698 | 16405874 | 22 | 2.98E-08 | 5.23E-05 |
| 22 | ENSG00000170893 | TRH | Thyrotropin-releasing hormone | 3 | 129693148 | 129696781 | 30 | 4.03E-08 | 6.81E-05 |
| 23 | ENSG00000199916 | RMRP | RNA component of mitochondrial RNA processing | 9 | 35657751 | 35658014 | 13 | 5.10E-08 | 8.02E-05 |
| 24 | ENSG00000223542 | RP1-283K11.3 | | 6 | 133756215 | 133760602 | 21 | 5.41E-08 | 8.23E-05 |
| 25 | ENSG00000257395 | RP11-597A11.2 | | 14 | 20149579 | 20152898 | 24 | 6.60E-08 | 9.40E-05 |
| 26 | ENSG00000258363 | RP11-146E13.6 | | 14 | 19959517 | 19961162 | 12 | 7.14E-08 | 9.78E-05 |
| 27 | ENSG00000121742 | GJB6 | Gap junction protein, beta 6, 30kDa | 13 | 20796110 | 20806534 | 13 | 7.29E-08 | 9.78E-05 |
| 28 | ENSG00000184115 | RP13-1039J1.2 | | 2 | 111143988 | 111192743 | 23 | 7.89E-08 | 0.00010277 |
| 29 | ENSG00000237754 | RP11-521C10.1 | | 7 | 67706624 | 67707254 | 17 | 1.11E-07 | 0.00014077 |
| 30 | ENSG00000225255 | LA16c-83F12.6 | | 22 | 16199666 | 16231333 | 11 | 1.45E-07 | 0.0001694 |
| 31 | ENSG00000265971 | RP11-269G24.6 | | 17 | 61532354 | 61533178 | 15 | 1.49E-07 | 0.0001694 |
| 32 | ENSG00000257731 | RP11-536C10.11 | | 14 | 19421521 | 19422698 | 14 | 1.82E-07 | 0.00019736 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 33 | ENSG00000157884 | CIB4 | Calcium and integrin binding family member 4 | 2 | 26804070 | 26864236 | 12 | 2.15E-07 | 0.00022269 |
| 34 | ENSG00000217889 | RP11-66N11.5 | | X | 152869952 | 152870803 | 25 | 2.57E-07 | 0.00026011 |
| 35 | ENSG00000100121 | GGTLC2 | Gamma-glutamyltransferase light chain 2 | 22 | 22988780 | 22990368 | 12 | 2.90E-07 | 0.00028768 |
| 36 | ENSG00000259671 | RP11-925D8.6 | | 15 | 58447159 | 58448260 | 46 | 4.25E-07 | 0.00041262 |
| 37 | ENSG00000232783 | AC073135.3 | | 3 | 197836983 | 197838749 | 37 | 6.65E-07 | 0.00059474 |
| 38 | ENSG00000225383 | SFTA1P | Surfactant associated 1, pseudogene | 10 | 10826400 | 10836943 | 23 | 7.16E-07 | 0.00062773 |
| 39 | ENSG00000224371 | RP11-235G24.1 | | 6 | 161293920 | 161339681 | 18 | 8.21E-07 | 0.0007017 |
| 40 | ENSG00000224758 | RP13-137A17.5 | | 10 | 134774844 | 134775741 | 17 | 8.31E-07 | 0.0007017 |
| 41 | ENSG00000203697 | CAPN8 | Calpain 8 | 1 | 223711349 | 223853436 | 9 | 8.99E-07 | 0.00074559 |
| 42 | ENSG00000257504 | RP11-536C10.7 | | 9 | 111899167 | 111929571 | 10 | 1.02E-06 | 0.00081855 |
| 43 | ENSG00000188536 | HBA2 | Hemoglobin, alpha 2 | 16 | 222846 | 223709 | 9 | 1.02E-06 | 0.00081855 |
| 44 | ENSG00000236045 | RP3-467K16.7 | | 9 | 113006091 | 113018920 | 10 | 1.59E-06 | 0.00117818 |
| 45 | ENSG00000238245 | MYO5BP2 | Myosin VB pseudogene 2 | 9 | 131217466 | 131263239 | 29 | 1.63E-06 | 0.00117818 |
| 46 | ENSG00000228897 | CTD-2021A8.2 | | 7 | 51454060 | 51454798 | 28 | 1.63E-06 | 0.00117818 |
| 47 | ENSG00000257608 | CTD-2311B13.6 | | 9 | 114122972 | 114247025 | 9 | 1.66E-06 | 0.00117836 |
| 48 | ENSG00000267242 | AC069278.4 | | 19 | 44978645 | 44979403 | 9 | 1.73E-06 | 0.00119303 |
| 49 | ENSG00000183091 | NEB | Nebulin | 9 | 132565432 | 132573560 | 9 | 1.84E-06 | 0.00123251 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 50 | ENSG00000222375 | RN7SKP127 | RNA, 7SK small nuclear pseudogene 127 | 16 | 29742372 | 29742725 | 9 | 1.84E-06 | 0.00123251 |
| 51 | ENSG00000234567 | RP1-283K11.2 | | 6 | 133773995 | 133777743 | 12 | 1.87E-06 | 0.00123251 |
| 52 | ENSG00000202198 | RN7SK | RNA, 7SK small nuclear | 9 | 132589569 | 132598142 | 9 | 2.18E-06 | 0.00142299 |
| 53 | ENSG00000233845 | AC093732.1 | | 2 | 47262418 | 47267663 | 16 | 2.26E-06 | 0.00144967 |
| 54 | ENSG00000230223 | ATXN8OS | ATXN8 opposite strand (non-protein coding) | 13 | 70681345 | 70705678 | 13 | 2.69E-06 | 0.00170472 |
| 55 | ENSG00000206172 | HBA1 | Hemoglobin, alpha 1 | 16 | 226679 | 227521 | 9 | 2.93E-06 | 0.00182928 |
| 56 | ENSG00000237674 | GSTA7P | Glutathione S-transferase alpha 7, pseudogene | 6 | 52604388 | 52609454 | 16 | 3.12E-06 | 0.00192314 |
| 57 | ENSG00000163618 | CADPS | Ca++-dependent secretion activator | 3 | 62384022 | 62861054 | 8 | 3.27E-06 | 0.00196311 |
| 58 | ENSG00000226958 | RNA28S5 | RNA, 28S ribosomal 5 | X | 108297361 | 108297792 | 8 | 3.41E-06 | 0.00201261 |
| 59 | ENSG00000260581 | CTB-113P19.4 | | 5 | 151031836 | 151035010 | 9 | 3.44E-06 | 0.00201261 |
| 60 | ENSG00000225649 | AC064875.2 | | 2 | 13106910 | 13147138 | 8 | 4.36E-06 | 0.00242637 |
| 61 | ENSG00000150361 | KLHL1 | Kelch-like family member 1 | 13 | 70274726 | 70682591 | 9 | 4.84E-06 | 0.00263214 |
| 62 | ENSG00000261760 | RP11-1223D19.1 | | 2 | 110988683 | 111043433 | 82 | 5.22E-06 | 0.00280248 |
| 63 | ENSG00000233306 | TRGV2 | T cell receptor gamma variable 2 | 7 | 38402465 | 38403119 | 10 | 5.31E-06 | 0.00281554 |
| 64 | ENSG00000233408 | LA16c-23H5.4 | | 22 | 16417269 | 16420386 | 12 | 5.47E-06 | 0.0028683 |
| 65 | ENSG00000162344 | FGF19 | Fibroblast growth factor 19 | 11 | 69513000 | 69519410 | 14 | 8.28E-06 | 0.00410331 |
| 66 | ENSG00000238881 | SCARNA2 | Small Cajal body-specific RNA 2 | 1 | 109642815 | 109643234 | 8 | 9.34E-06 | 0.00458036 |
| 67 | ENSG00000258730 | ITPK1-AS1 | ITPK1 antisense RNA 1 | 14 | 93533797 | 93538497 | 17 | 9.87E-06 | 0.00478817 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 68 | ENSG00000211699 | TRGV3 | T cell receptor gamma variable 3 | 7 | 38398113 | 38398763 | 10 | 1.01E-05 | 0.004856 |
| 69 | ENSG00000260555 | RP11-728K20.2 | | 7 | 149697841 | 149702213 | 9 | 1.12E-05 | 0.00531157 |
| 70 | ENSG00000251060 | U66061.31 | | 7 | 142413074 | 142426272 | 10 | 1.33E-05 | 0.00607493 |
| 71 | ENSG00000225210 | AL589743.1 | | 14 | 19650018 | 19718563 | 7 | 1.40E-05 | 0.00633854 |
| 72 | ENSG00000211688 | TRGJP2 | T cell receptor gamma joining P2 | 7 | 38295938 | 38295997 | 10 | 1.46E-05 | 0.00654432 |
| 73 | ENSG00000244067 | GSTA2 | Glutathione S-transferase alpha 2 | 6 | 52614897 | 52628367 | 7 | 1.53E-05 | 0.00673148 |
| 74 | ENSG00000249170 | RP11-IJ11.1 | | 4 | 72165975 | 72166952 | 12 | 1.66E-05 | 0.00714847 |
| 75 | ENSG00000232079 | AL035610.1 | | 21 | 29420733 | 29509930 | 13 | 1.77E-05 | 0.00753353 |
| 76 | ENSG00000250984 | CTD-2011G17.1 | | 5 | 29796045 | 29796375 | 15 | 1.90E-05 | 0.00802856 |
| 77 | ENSG00000258265 | CTD-2311B13.2 | | 14 | 19529958 | 19530898 | 69 | 2.07E-05 | 0.00857855 |
| 78 | ENSG00000225258 | AC009478.1 | | 2 | 181436439 | 181557181 | 10 | 2.19E-05 | 0.00889804 |
| 79 | ENSG00000180532 | ZSCAN4 | Zinc finger and SCAN domain containing 4 | 19 | 58180303 | 58190520 | 7 | 2.43E-05 | 0.00978666 |
| 80 | ENSG00000138100 | TRIM54 | Tripartite motif containing 54 | 2 | 27505260 | 27530307 | 7 | 2.59E-05 | 0.01037866 |
| 81 | ENSG00000231565 | NEK2P2 | NEK2 pseudogene 2 | 22 | 16364867 | 16366204 | 7 | 2.66E-05 | 0.01053375 |
| 82 | ENSG00000227195 | RP3-410C9.1 | | 20 | 26167556 | 26232162 | 6 | 3.07E-05 | 0.01207635 |
| 83 | ENSG00000164093 | PITX2 | Paired-like homeodomain 2 | 4 | 111538579 | 111563279 | 8 | 3.11E-05 | 0.01211525 |
| 84 | ENSG00000169856 | ONECUT1 | One cut homeobox 1 | 15 | 53049186 | 53083273 | 65 | 3.38E-05 | 0.01296255 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 85 | ENSG00000214976 | VDAC2P1 | Voltage-dependent anion channel 2 pseudogene 1 | 21 | 17466735 | 17467692 | 62 | 3.38E-05 | 0.01296255 |
| 86 | ENSG00000117154 | IGSF21 | Immunoglobulin superfamily, member 21 | 1 | 18434240 | 18704977 | 6 | 3.77E-05 | 0.01425025 |
| 87 | ENSG00000166748 | AGBL1 | ATP/GTP binding protein-like 1 | 15 | 86685227 | 87572283 | 8 | 3.78E-05 | 0.01425025 |
| 88 | ENSG00000239736 | CEACAMP3 | Carcinoembryonic antigen-related cell adhesion | 19 | 42106090 | 42112339 | 20 | 3.82E-05 | 0.01426299 |
| 89 | ENSG00000204038 | AL359195.1 | Uncharacterized protein; cDNA FLJ46261 fis, clone | 10 | 82009466 | 82013395 | 27 | 3.99E-05 | 0.01467239 |
| 90 | ENSG00000206177 | HBM | Hemoglobin, mu | 16 | 203891 | 216767 | 27 | 3.99E-05 | 0.01467239 |
| 91 | ENSG00000267614 | AC138472.6 | | 19 | 45040251 | 45040599 | 11 | 4.45E-05 | 0.01622454 |
| 92 | ENSG00000259425 | RP11-566K19.5 | | 15 | 23096869 | 23105332 | 7 | 4.99E-05 | 0.01763448 |
| 93 | ENSG00000228477 | RP3-342P20.2 | | 1 | 40428352 | 40429076 | 9 | 5.34E-05 | 0.01874722 |
| 94 | ENSG00000254127 | IGLCOR22-1 | Immunoglobulin lambda constant/OR22-1 | 22 | 32595906 | 32596221 | 14 | 5.40E-05 | 0.01880063 |
| 95 | ENSG00000169427 | KCNK9 | Potassium channel, subfamily K, member 9 | 8 | 140613081 | 140715299 | 58 | 5.63E-05 | 0.01927405 |
| 96 | ENSG00000261426 | AC144833.1 | | 15 | 27607456 | 27611544 | 58 | 5.63E-05 | 0.01927405 |
| 97 | ENSG00000236714 | AC005592.1 | | 5 | 142125165 | 142140563 | 6 | 5.99E-05 | 0.02023964 |
| 98 | ENSG00000166816 | LDHD | Lactate dehydrogenase D | 16 | 75145758 | 75150669 | 6 | 6.06E-05 | 0.02033455 |
| 99 | ENSG00000178934 | LGALS7B | Lectin, galactoside-binding, soluble, 7B | 19 | 39279851 | 39282389 | 7 | 6.35E-05 | 0.02103095 |
| 100 | ENSG00000148826 | NKX6-2 | NK6 homeobox 2 | 10 | 134598297 | 134599556 | 20 | 6.37E-05 | 0.02103095 |
| 101 | ENSG00000244280 | ECEL1P2 | Endothelin converting enzyme-like 1, pseudogene 2 | 2 | 233250460 | 233252167 | 7 | 6.41E-05 | 0.02103095 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 102 | ENSG00000259344 | RP11-566K19.6 | | 15 | 23095170 | 23115256 | 6 | 6.57E-05 | 0.02138789 |
| 103 | ENSG00000253988 | RP11-489O18.1 | | 8 | 139075511 | 139085483 | 8 | 6.99E-05 | 0.0226057 |
| 104 | ENSG00000255193 | RP11-945A11.1 | | 11 | 23752134 | 23818516 | 7 | 7.06E-05 | 0.02268337 |
| 105 | ENSG00000256642 | LINC00273 | Long intergenic non-protein coding RNA 273 | 16 | 33961052 | 33962503 | 6 | 7.40E-05 | 0.02311437 |
| 106 | ENSG00000173809 | TDRD12 | Tudor domain containing 12 | 19 | 33210659 | 33320483 | 6 | 7.63E-05 | 0.02366584 |
| 107 | ENSG00000248408 | RP11-452C8.1 | | 4 | 80584915 | 80617991 | 6 | 7.72E-05 | 0.02366754 |
| 108 | ENSG00000109158 | GABRA4 | Gamma-aminobutyric acid (GABA) A receptor, alpha | 4 | 46920917 | 46996424 | 13 | 7.79E-05 | 0.02366754 |
| 109 | ENSG00000261467 | RP11-731K22.1 | | 7 | 73400322 | 73403097 | 13 | 7.79E-05 | 0.02366754 |
| 110 | ENSG00000234232 | RP11-353N4.5 | | 1 | 149691432 | 149698605 | 24 | 9.34E-05 | 0.02820155 |
| 111 | ENSG00000152969 | JAKMIP1 | Janus kinase and microtubule interacting | 4 | 6027926 | 6202318 | 6 | 9.95E-05 | 0.02975485 |
| 112 | ENSG00000257898 | | | | | | 6 | 9.98E-05 | 0.02975485 |
| 113 | ENSG00000142619 | PADI3 | Peptidyl arginine deiminase, type III | 1 | 17575593 | 17610728 | 6 | 0.00010415 | 0.03083656 |
| 114 | ENSG00000264462 | MIR3648 | MicroRNA 3648 | 21 | 9825832 | 9826011 | 6 | 0.00010575 | 0.03111075 |
| 115 | ENSG00000260284 | TPSP2 | Tryptase pseudogene 2 | 16 | 1336352 | 1338838 | 6 | 0.00010657 | 0.03115014 |
| 116 | ENSG00000236138 | RP11-413E6.7 | | 3 | 75718082 | 75719336 | 7 | 0.00011147 | 0.0323757 |
| 117 | ENSG00000230666 | CEACAM22P | Carcinoembryonic antigen-related cell adhesion | 19 | 45051045 | 45124119 | 6 | 0.00011558 | 0.03314582 |
| 118 | ENSG00000257884 | RP11-597A11.4 | | 14 | 20145996 | 20148263 | 6 | 0.00012295 | 0.03461735 |
| 119 | ENSG00000235994 | RP3-470B24.5 | | 6 | 169376604 | 168380456 | 11 | 0.00012299 | 0.03461735 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 120 | ENSG00000205076 | LGALS7 | Lectin, galactoside-binding, soluble, 7 | 19 | 39261611 | 39264132 | 11 | 0.00012299 | 0.03461735 |
| 121 | ENSG00000252139 | SCARNA18 | Small Cajal body-specific RNA 18 | 18 | 47340731 | 47340813 | 15 | 0.00012697 | 0.03551893 |
| 122 | ENSG00000180383 | DEFB124 | Defensin, beta 124 | 20 | 30053309 | 30064560 | 7 | 0.00012856 | 0.0357452 |
| 123 | ENSG00000170509 | HSD17B13 | Hydroxysteroid (17-beta) dehydrogenase 13 | 4 | 88224941 | 88244058 | 6 | 0.00013281 | 0.03648018 |
| 124 | ENSG00000132204 | LINC00470 | Long intergenic non-protein coding RNA 470 | 18 | 1254384 | 1408345 | 7 | 0.00013723 | 0.03746994 |
| 125 | ENSG00000237686 | RP5-1120P11.1 | | 6 | 43963460 | 44042389 | 6 | 0.00014396 | 0.03907418 |
| 126 | ENSG00000253326 | RP11-261C10.7 | | 1 | 243218163 | 243219696 | 23 | 0.00014577 | 0.03933051 |
| 127 | ENSG00000259600 | RP11-925D8.3 | | 15 | 58443232 | 58444712 | 8 | 0.00015274 | 0.04072871 |
| 128 | ENSG00000206195 | AP000525.9 | | 22 | 16147979 | 16193004 | 6 | 0.0001548 | 0.04103847 |
| 129 | ENSG00000266998 | RP11-936I5.1 | | 17 | 75369900 | 75373318 | 9 | 0.00016348 | 0.04284121 |
| 130 | ENSG00000221673 | U3 | Small nucleolar RNA U3 | 1 | 220136028 | 220136206 | 49 | 0.00016596 | 0.04299561 |
| 131 | ENSG00000240881 | RP11-713P14.1 | | 11 | 23541868 | 23542748 | 49 | 0.00016596 | 0.04299561 |
| 132 | ENSG00000170423 | KRT78 | Keratin 78 | 12 | 53231588 | 53242876 | 10 | 0.00016848 | 0.04315965 |
| 133 | ENSG00000166268 | MYRFL | Myelin regulatory factor-like | 12 | 70219084 | 70352877 | 6 | 0.0001709 | 0.04353335 |
| 134 | ENSG00000259787 | CTB-85P21.2 | | 5 | 143861871 | 143865249 | 10 | 0.0001722 | 0.04353335 |
| 135 | ENSG00000263015 | RP5-1029F21.3 | | 17 | 406411 | 414587 | 11 | 0.0001728 | 0.04353335 |
| 136 | ENSG00000223764 | RP11-54O7.3 | | 1 | 852250 | 855072 | 5 | 0.00019537 | 0.04815425 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 12.** Differentially expressed genes (FDR < 0.05) up-regulated by soy isoflavone supplementation of the 166 differentially expressed genes from only normal tissue obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ENSG00000206073 | SERPINB4 | Serpin peptidase inhibitor, clade B (ovalbumin), member 4 | 18 | 61304493 | 61311532 | 69 | 7.13E-10 | 2.50E-06 |
| 2 | ENSG00000057149 | SERPINB3 | Serpin peptidase inhibitor, clade B (ovalbumin), member 3 | 18 | 61322431 | 61329197 | 32 | 1.33E-07 | 0.000158996 |
| 3 | ENSG00000264209 | AC104448.1 | | 3 | 48529745 | 48529838 | 266 | 6.30E-07 | 0.000586159 |
| 4 | ENSG00000267056 | AC005336.4 | | 19 | 16021393 | 16022634 | 18 | 1.49E-06 | 0.001134641 |
| 5 | ENSG00000175445 | LPL | Lipoprotein lipase | 9 | 19759228 | 19824769 | 16 | 3.25E-06 | 0.001963106 |
| 6 | ENSG00000163735 | CXCL5 | Chemokine (C-X-C motif) ligand 5 | 4 | 74861359 | 74864496 | 16 | 3.54E-06 | 0.002038721 |
| 7 | ENSG00000182816 | KRTAP13-2 | Keratin associated protein 13-2 | 21 | 31743709 | 31744557 | 40 | 3.58E-06 | 0.002038721 |
| 8 | ENSG00000163209 | SPRR3 | Small proline-rich protein 3 | 1 | 152974223 | 152976332 | 29 | 4.85E-06 | 0.002632142 |
| 9 | ENSG00000000005 | TNMD | Tenomodulin | X | 99839799 | 99854882 | 18 | 7.34E-06 | 0.003746265 |
| 10 | ENSG00000184937 | WT1 | Wilms tumor 1 | 11 | 32409321 | 32457176 | 15 | 7.39E-06 | 0.003746265 |
| 11 | ENSG00000251165 | RP11-215A19.1 | | 4 | 187207248 | 187422151 | 49 | 7.58E-06 | 0.003795706 |
| 12 | ENSG00000134339 | SAA2 | Serum amyloid A2 | 11 | 18260770 | 18270190 | 13 | 1.14E-05 | 0.005357755 |
| 13 | ENSG00000227063 | RPL41P1 | Ribosomal protein L41 pseudogene 1 | 20 | 21735866 | 21736171 | 17 | 1.20E-05 | 0.005515008 |
| 14 | ENSG00000243063 | IGKV3-7 | Immunoglobulin kappa variable 3-7 (non-functional) | 2 | 89277987 | 89278600 | 161 | 1.55E-05 | 0.00673148 |
| 15 | ENSG00000143556 | S100A7 | S100 calcium binding protein A7 | 1 | 153430220 | 153433177 | 155 | 1.55E-05 | 0.00673148 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | ENSG00000211974 | IGHV2-70 | Immunoglobulin heavy variable 2-70 | 14 | 107178820 | 107179338 | 13 | 1.93E-05 | 0.008055596 |
| 17 | ENSG00000198535 | C2CD4A | C2 calcium-dependent domain containing 4A | 15 | 62359176 | 62363116 | 12 | 2.17E-05 | 0.008898036 |
| 18 | ENSG00000099290 | FAM21A | Family with sequence similarity 21, member A | 10 | 51827648 | 51893269 | 11 | 4.90E-05 | 0.017634483 |
| 19 | ENSG00000231083 | AC011747.6 | | 2 | 8741022 | 8763072 | 36 | 4.93E-05 | 0.017634483 |
| 20 | ENSG00000196427 | NBPF4 | Neuroblastoma breakpoint family, member 4 | 1 | 108765087 | 108786689 | 130 | 4.98E-05 | 0.017634483 |
| 21 | ENSG00000104267 | CA2 | Carbonic anhydrase II | 8 | 86376081 | 86393722 | 10 | 5.66E-05 | 0.019274052 |
| 22 | ENSG00000198178 | CLEC4C | C-type lectin domain family 4, member C | 12 | 7882011 | 7904201 | 116 | 7.22E-05 | 0.023032885 |
| 23 | ENSG00000229403 | RP4-718N17.2 | | 7 | 51681947 | 51698566 | 15 | 7.36E-05 | 0.023114368 |
| 24 | ENSG00000069206 | ADAM7 | ADAM metallopeptidase domain 7 | 8 | 24298443 | 24384483 | 13 | 0.00011541 | 0.03314582 |
| 25 | ENSG00000104760 | FGL1 | Fibrinogen-like 1 | 8 | 17721889 | 17767874 | 12 | 0.00013199 | 0.036475647 |
| 26 | ENSG00000095917 | TPSD1 | Tryptase delta 1 | 16 | 1306060 | 1308532 | 10 | 0.000149058 | 0.039980789 |
| 27 | ENSG00000166396 | SERPINB7 | Serpin peptidase inhibitor, clade B (ovalbumin), member 7 | 18 | 61420169 | 61472604 | 10 | 0.00015935 | 0.042000137 |
| 28 | ENSG00000119913 | TECTB | Tectorin beta | 10 | 114043493 | 114064793 | 11 | 0.000168324 | 0.043159648 |
| 29 | ENSG00000164756 | SLC30A8 | Solute carrier family 30 (zinc transporter), member 8 | 8 | 117962512 | 118188953 | 17 | 0.000185505 | 0.045970975 |
| 30 | ENSG00000244921 | CTB-36O1.7 | | 5 | 134258993 | 134259739 | 9 | 0.000201827 | 0.049478048 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 13.** Differentially expressed genes (FDR < 0.05) present in both normal and tumor tissues obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | | | | | | | | Normal tissue | | | Tumor tissue | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR | Fold change | p-value | FDR |
| 1 | ENSG00000254851 | RP11-109L13.1 | | 11 | 117006244 | 117009298 | -16 | 1.90E-07 | 0.000201776 | -340 | 4.88E-14 | 5.11E-10 |
| 2 | ENSG00000164266 | SPINK1 | Serine peptidase inhibitor, Kazal type 1 | 5 | 147204131 | 147211349 | 63 | 1.01E-09 | 3.28E-06 | 518 | 9.04E-11 | 5.41E-07 |
| 3 | ENSG00000258027 | RP11-597A11.1 | | 14 | 20078033 | 20146082 | -22 | 4.68E-08 | 7.63E-05 | -31 | 2.20E-08 | 5.76E-05 |
| 4 | ENSG00000121075 | TBX4 | T-box 4 | 17 | 59529765 | 59562471 | 9 | 0.000180519 | 0.045226933 | -23 | 8.97E-08 | 0.000170712 |
| 5 | ENSG00000253998 | IGKV2-29 | Immunoglobulin kappa variable 2-29 | 2 | 89533655 | 89534393 | -40 | 3.89E-06 | 0.002192593 | -174 | 1.35E-07 | 0.000234152 |
| 6 | ENSG00000171564 | FGB | Fibrinogen beta chain | 4 | 155484108 | 155492238 | 67 | 1.11E-06 | 0.000869858 | 222 | 2.78E-07 | 0.000431338 |
| 7 | ENSG00000257357 | RP11-244H18.3 | | 14 | 20105463 | 20109540 | -23 | 5.50E-07 | 0.000522509 | -24 | 5.87E-07 | 0.00072277 |
| 8 | ENSG00000233760 | AC004947.2 | | 7 | 26591441 | 26596819 | 11 | 7.32E-05 | 0.023114368 | 68 | 5.90E-07 | 0.00072277 |
| 9 | ENSG00000260125 | RP11-31E22.1 | | 15 | 86838880 | 86860404 | -44 | 5.47E-11 | 3.56E-07 | -40 | 2.03E-06 | 0.0018495 |
| 10 | ENSG00000170369 | CST2 | Cystatin SA | 20 | 23804406 | 23807368 | 27 | 1.15E-07 | 0.000141837 | 45 | 2.56E-06 | 0.002189989 |
| 11 | ENSG00000096006 | CRISP3 | Cysteine-rich secretory protein 3 | 6 | 49695097 | 49712150 | -12 | 6.41E-08 | 9.40E-05 | 44 | 3.69E-06 | 0.00302595 |
| 12 | ENSG00000226278 | PSPHP1 | Phosphoserine phosphatase pseudogene 1 | 7 | 55832490 | 55840981 | 100 | 8.43E-09 | 1.92E-05 | 116 | 4.65E-06 | 0.003602916 |
| 13 | ENSG00000156096 | UGT2B4 | UDP glucurosyltransferase 2 family, polypeptide B4 | 4 | 70345883 | 70391732 | 26 | 1.68E-06 | 0.001178363 | 28 | 4.06E-05 | 0.020883677 |
| 14 | ENSG00000188000 | OR7D2 | Olfactory receptor, family 7, subfamily D, member 2 | 19 | 9296279 | 9299493 | -7 | 0.000182463 | 0.045464119 | -14 | 4.09E-05 | 0.020883677 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | ENSG00000172238 | ATOH1 | Atonal homolog 1 (Drosophila) | 4 | 94750042 | 94751221 | 22 | 1.15E-05 | 0.005357755 | 216 | 4.63E-05 | 0.021798893 |
| 16 | ENSG00000234665 | RP11-262H14.3 | | 9 | 66513488 | 66553911 | -19 | 6.65E-07 | 0.000594735 | -14 | 5.86E-05 | 0.026385604 |
| 17 | ENSG00000234931 | MARK2P15 | MAP/microtubule affinity-regulating kinase 2 pseudogene 15 | 10 | 85071384 | 85074231 | -61 | 1.53E-13 | 3.49E-09 | -29 | 7.24E-05 | 0.030023263 |
| 18 | ENSG00000170356 | OR2A20P | Olfactory receptor, family 2, subfamily A, member 20 pseudogene | 7 | 143947138 | 143950050 | -11 | 6.71E-06 | 0.003474592 | -12 | 0.000100736 | 0.03698971 |
| 19 | ENSG00000247627 | MTND4P12 | MT-ND4 pseudogene 12 | 5 | 134262350 | 134263726 | 19 | 1.13E-06 | 0.000869858 | 18 | 0.000114525 | 0.040285875 |
| 20 | ENSG00000259761 | RP11-138H10.2 | | 15 | 87585285 | 87590392 | -119 | 1.65E-07 | 0.000183268 | -64 | 0.000140283 | 0.046604968 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 14.** Differentially expressed genes (FDR < 0.05) present in and differentially regulated by soy isoflavone supplementation in normal tissue compared to tumor tissues obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | | | | | | | | Normal tissue | | | Tumor tissue | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR | Fold change | p-value | FDR |
| 1 | ENSG00000121075 | TBX4 | T-box 4 | 17 | 59529765 | 59562471 | 9 | 0.000180519 | 0.045226933 | -23 | 8.97E-08 | 0.000170712 |
| 2 | ENSG00000096006 | CRISP3 | Cysteine-rich secretory protein 3 | 6 | 49695097 | 49712150 | -12 | 6.41E-08 | 9.40E-05 | 44 | 3.69E-06 | 0.00302595 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 15.** Differentially expressed genes (FDR < 0.05) down-regulated by soy isoflavone supplementation of the 20 differentially expressed genes present in both normal and tumor tissues obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | | | | | | | | Normal tissue | | Tumor tissue | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR | Fold change | p-value | FDR |
| 1 | ENSG00000164266 | SPINK1 | Serine peptidase inhibitor, Kazal type 1 | 5 | 147204131 | 147211349 | 63 | 1.01E-09 | 3.28E-06 | 518 | 9.04E-11 | 5.41E-07 |
| 2 | ENSG00000171564 | FGB | Fibrinogen beta chain | 4 | 155484108 | 155492238 | 67 | 1.11E-06 | 0.000869858 | 222 | 2.78E-07 | 0.000431338 |
| 3 | ENSG00000233760 | AC004947.2 | | 7 | 26591441 | 26596819 | 11 | 7.32E-05 | 0.023114368 | 68 | 5.90E-07 | 0.00072277 |
| 4 | ENSG00000170369 | CST2 | Cystatin SA | 20 | 23804406 | 23807368 | 27 | 1.15E-07 | 0.000141837 | 45 | 2.56E-06 | 0.002189989 |
| 5 | ENSG00000226278 | PSPHP1 | Phosphoserine phosphatase pseudogene 1 | 7 | 55832490 | 55840981 | 100 | 8.43E-09 | 1.92E-05 | 116 | 4.65E-06 | 0.003602916 |
| 6 | ENSG00000156096 | UGT2B4 | UDP glucuronosyltransferase 2 family, polypeptide | 4 | 70345883 | 70391732 | 26 | 1.68E-06 | 0.001178363 | 28 | 4.06E-05 | 0.020883677 |
| 7 | ENSG00000172238 | ATOH1 | Atonal homolog 1 (Drosophila) | 4 | 94750042 | 94751221 | 22 | 1.15E-05 | 0.005357755 | 216 | 4.63E-05 | 0.021798893 |
| 8 | ENSG00000247627 | MNTD4P12 | MT-ND4 pseudogene 12 | 5 | 134262350 | 134263726 | 19 | 1.13E-06 | 0.000869858 | 18 | 0.000114525 | 0.040285875 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 16.** Differentially expressed genes (FDR < 0.05) up-regulated by soy isoflavone supplemention of the 20 differentially expressed genes present in both normal and tumor tissues obtained from 4 men who consumed soy isoflavones and 2 who consumed placebo (n = 6)

| | | | | | | | | Normal tissue | | Tumor tissue | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gene ID | Gene abbreviation | Gene name | Chr | BP Start | BP End | Fold change | p-value | FDR | Fold change | p-value | FDR |
| 1 | ENSG00000254851 | RP11-109L13.1 | | 11 | 117006244 | 117009298 | -16 | 1.90E-07 | 0.000201776 | -340 | 4.88E-14 | 5.11E-10 |
| 2 | ENSG00000258027 | RP11-597A11.1 | | 14 | 20078033 | 20146082 | -22 | 4.68E-08 | 7.63E-05 | -31 | 2.20E-08 | 5.76E-05 |
| 3 | ENSG00000253998 | IGKV2-29 | Immunoglobulin kappa variable 2-29 | 2 | 89533655 | 89534393 | -40 | 3.89E-06 | 0.002192593 | -174 | 1.35E-07 | 0.000234152 |
| 4 | ENSG00000257357 | RP11-244H18.3 | | 14 | 20105463 | 20109540 | -23 | 5.50E-07 | 0.000522509 | -24 | 5.87E-07 | 0.00072277 |
| 5 | ENSG00000260125 | RP11-31E22.1 | | 15 | 86838880 | 86860404 | -44 | 5.47E-11 | 3.56E-07 | -40 | 2.03E-06 | 0.0018495 |
| 6 | ENSG00000188000 | OR7D2 | Olfactory receptor, family 7, subfamily D, member 2 | 19 | 9296279 | 9299493 | -7 | 0.000182463 | 0.045464119 | -14 | 4.09E-05 | 0.020883677 |
| 7 | ENSG00000234665 | RP11-262H14.3 | | 9 | 66513488 | 66553911 | -19 | 6.65E-07 | 0.000594735 | -14 | 5.86E-05 | 0.026385604 |
| 8 | ENSG00000234931 | MARK2P15 | MAP/microtubule affinity-regulating kinase 2 pseudogene 15 | 10 | 85071384 | 85074231 | -61 | 1.53E-13 | 3.49E-09 | -29 | 7.24E-05 | 0.030023263 |
| 9 | ENSG00000170356 | OR2A20P | Olfactory receptor, family 2, subfamily A, member 20 pseudogene | 7 | 143947138 | 143950050 | -11 | 6.71E-06 | 0.003474592 | -12 | 0.000100736 | 0.03698971 |
| 10 | ENSG00000259761 | RP11-138H10.2 | | 15 | 87585285 | 87590392 | -119 | 1.65E-07 | 0.000183268 | -64 | 0.000140283 | 0.046604968 |

Chr, chromosome; BP, base pair; FDR, false discovery rate

**Table 17. Top 5 significantly enriched canonical pathways expressed in tumor tissue**

| | Canonical Pathways | -log(p-value) | Ratio | Molecules |
|---|---|---|---|---|
| 1 | Extrinsic prothrombin activation pathway | 2.59E00 | 1.25E-01 | FGB, FGA |
| 2 | Human embryonic stem cell pluripotency | 2.44E00 | 2.68E-02 | NANOG, BMP5, ZIC3, WNT2 |
| 3 | Intrinisic prothrombin activation pathway | 2.11E00 | 6.25E-02 | FGB, FGA |
| 4 | Acute phase response signaling | 2.08E00 | 2.31E-02 | FGB, LBP, FGA, RBP4 |
| 5 | Complement system | 2E00 | 6.06E-02 | CFD, CR2 |

**Table 18. Top 5 significantly enriched canonical pathways expressed in normal tissue**

| | Canonical Pathways | -log(p-value) | Ratio | Molecules |
|---|---|---|---|---|
| 1 | Role of IL-17A in psoriasis | 2.96E00 | 1.54E-01 | S100A7, CXCL5 |
| 2 | Methylglyoxal degradation VI | 2.42E00 | 1E00 | LDHD |
| 3 | Glutamate dependent acid resistance | 2.12E00 | 5E-01 | GAD2 |
| 4 | GABA receptor signaling | 1.87E00 | 4.35E-02 | GAD2, GABRA4 |
| 5 | Protein citrullination | 1.72E00 | 2E-01 | PADI3 |

**Table 19. Top 5 significantly enriched canonical pathways expressed in both normal and tumor tissues**

| | Canonical Pathways | -log(p-value) | Ratio | Molecules |
|---|---|---|---|---|
| 1 | Extrinsic prothrombin activation pathway | 2.14E00 | 6.25E-02 | FGB |
| 2 | Thyroid hormone metabolism II | 1.93E00 | 2.85E-02 | UGT2B4 |
| 3 | Intrinisic prothrombin activation pathway | 1.9E00 | 3.12E-02 | FGB |
| 4 | Coagulation system | 1.8E00 | 2.86E-02 | FGB |
| 5 | Nicotine degradation pathway III | 1.69E00 | 2.22E-02 | UGT2B4 |

**Table 20. Specific genes expressed within the top 5 significantly enriched canonical pathways expressed in normal tissue, tumor tissue, and both tissues**

| | | Normal tissue | | | Tumor tissue | | |
|---|---|---|---|---|---|---|---|
| **Genes** | **Canonical pathways** | **Log ratio** | **p-value** | **FDR** | **Log ratio** | **p-value** | **FDR** |
| UGT2B4 | Thyroid hormone metabolism II<br>Nicotine degradation III | + 4.717 | 1.68E-06 | 1.18E-03 | + 4.784 | 4.06E-05 | 2.09E-02 |
| FGB | Extrinsic prothrombin activation<br>Intrinsic prothrombin activation<br>Acute phase response signaling<br>Coagulation system | + 6.076 | 1.11E-06 | 8.70E-04 | + 7.795 | 2.78E-07 | 4.31E-04 |
| S100A7 | Role of IL-17A in psoriasis | +7.276 | 1.55E-05 | 6.73E-03 | | | |
| CXCL5 | Role of IL-17A in psoriasis | +3.972 | 3.54E-06 | 2.04E-03 | | | |
| LDHD | Methylglyoxal degradation VI | -2.592 | 6.06E-05 | 2.03E-02 | | | |
| GAD2 | Glutamate dependent acid resistance<br>GABA receptor signaling | -6.378 | 2.92E-14 | 1.33E-09 | | | |
| GABRA4 | GABA receptor signaling | -3.731 | 7.79E-05 | 2.37E-02 | | | |
| PADI3 | Protein citrullination | -2.546 | 1.04E-04 | 3.08E-02 | | | |
| FGA | Extrinsic prothrombin activation<br>Intrinsic prothrombin activation<br>Acute phase response signaling | | | | +7.814 | 4.28E-05 | 2.13E-02 |
| ZIC3 | Human embryonic stem cell pluripotency | | | | +5.719 | 1.29E-04 | 4.40E-02 |
| NANOG | Human embryonic stem cell pluripotency | | | | -5.962 | 1.40E-04 | 4.66E-02 |
| BMP5 | Human embryonic stem cell pluripotency | | | | -7.872 | 6.66E-16 | 2.79E-11 |
| WNT2 | Human embryonic stem cell pluripotency | | | | -3.640 | 9.60E-06 | 6.48E-03 |
| LBP | Acute phase response signaling | | | | -3.466 | 1.07E-04 | 3.85E-02 |

| | | | | |
|---|---|---|---|---|
| RBP4 | Acute phase response signaling | -4.010 | 1.46E-06 | 1.45E-03 |
| CFD | Complement system | -3.151 | 9.99E-05 | 6.28E-04 |
| CR2 | Complement system | -4.850 | 2.54E-08 | 6.25E-05 |

FDR, false discovery rate

**Table 21. Clinical outcomes for study sample**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Patient** | 1 | 2 | 3 | 4 | 5 | 6 |
| | **ID** | 07 | 34 | 36 | 37 | 38 | 50 |
| | **Treatment** | SOY | SOY | PLACEBO | SOY | PLACEBO | SOY |
| | **Gleason score** | 7 | 9 | 7 | 7 | 7 | 7 |
| | **Stage** | T2C N0 M0 | T3A N0 M0 | T2C N0 M0 | T2A N1 M0 | T2C N0 M0 | T2C N0 M0 |
| | **AJCC** | IIB | III | IIB | IIA | IIB | IIB |
| **Pathology** | **Margins** | NEG | NEG | NEG | NEG | NEG | POS |
| **Pathology** | **Seminal vesicle invasion** | NEG | NEG | NEG | NEG | NEG | NEG |
| **Pathology** | **Extracapsular extension** | NEG | POS | NEG | NEG | NEG | NEG |
| **Follow-up data** | **PSA (ng/mL)** | <0.04 | 821.3 | 0.00 | 0.04 | 0.00 | 0.00 |
| **Follow-up data** | **BCR** | NO | NO | NO | NO | NO | NO |
| **Follow-up data** | **Adjuvant treatment** | NONE | ADT/CHEMO | NONE | NONE | NONE | NONE |
| **Follow-up data** | **Salvage treatment** | NONE | NONE | NONE | NONE | NONE | NONE |
| **Follow-up data** | **Survival status** | ALIVE | DEAD | DEAD | ALIVE | ALIVE | ALIVE |
| **Follow-up data** | **Cause of death** | ALIVE | PROSTATE CANCER | UNKNOWN | ALIVE | ALIVE | ALIVE |

AJCC, American Joint Committee on Cancer; PSA, prostate specific antigen; BCR, biochemical recurrence

**Figure 2. Top 5 significantly enriched canonical pathways expressed in tumor tissue**

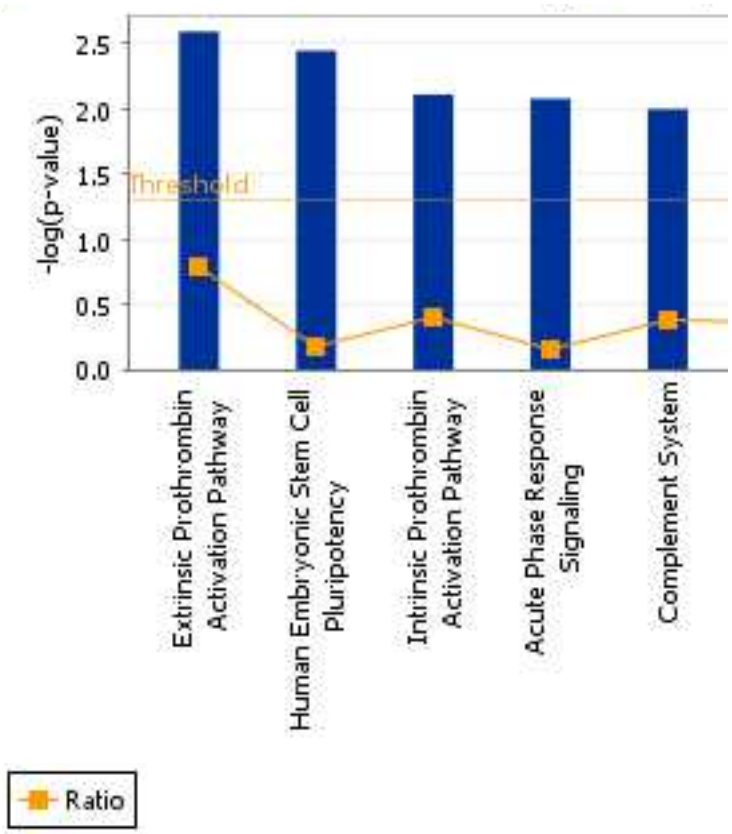


**Figure 3. Percentage of genes expressed in the dataset within the top 5 significantly enriched canonical pathways expressed in tumor tissue**

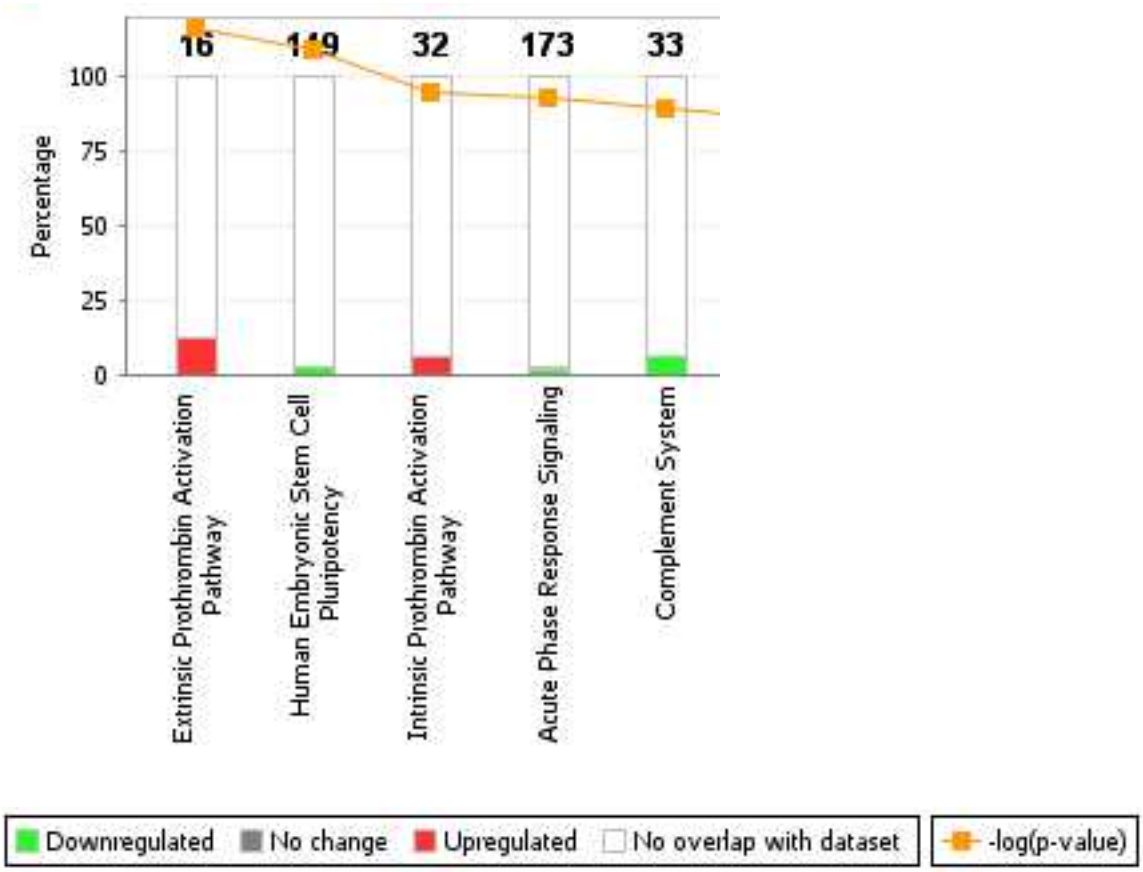


**Figure 4. Top 5 significantly enriched canonical pathways expressed in normal tissue**

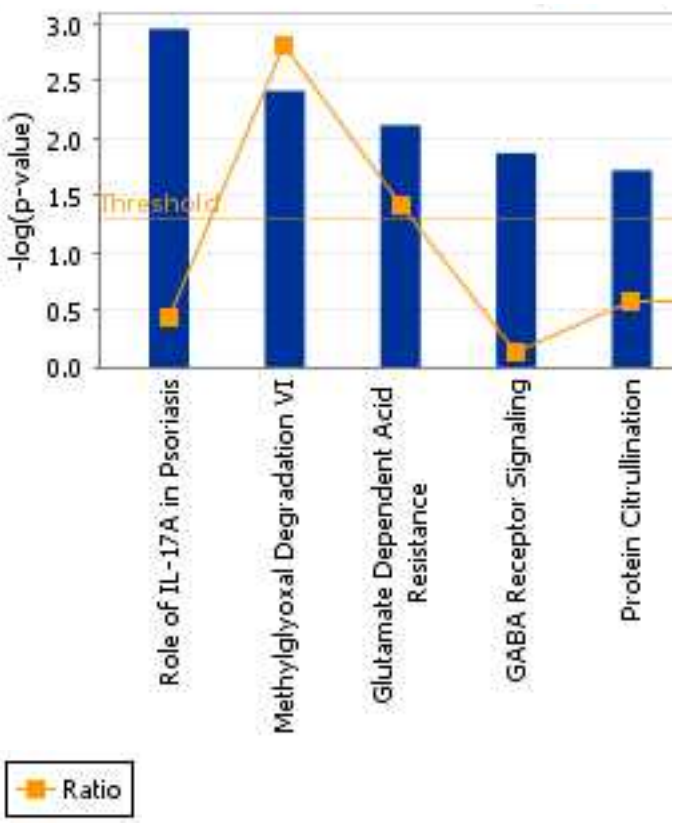


**Figure 5. Percentage of genes expressed in the dataset with in the top 5 significantly enriched canonical pathways expressed in normal tissue**

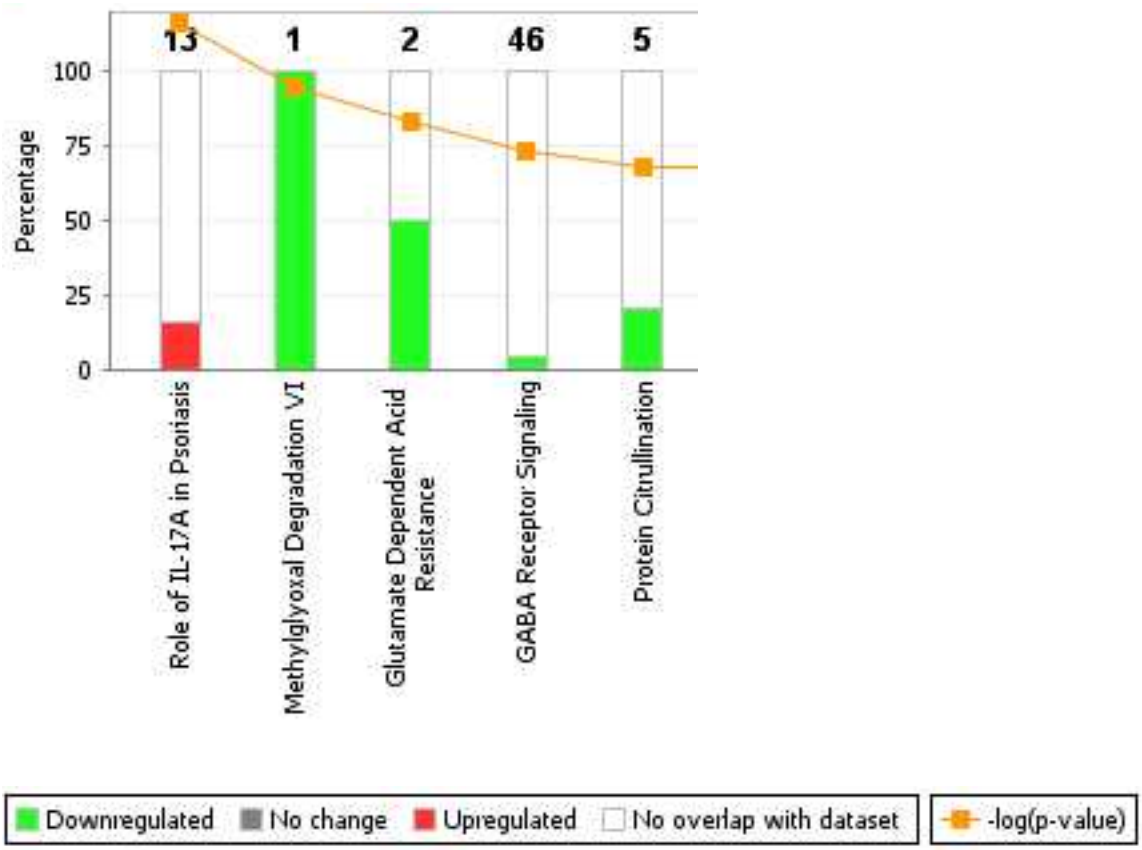


**Figure 6. Top 13 significantly enriched canonical pathways expressed in both normal and tumor tissues**

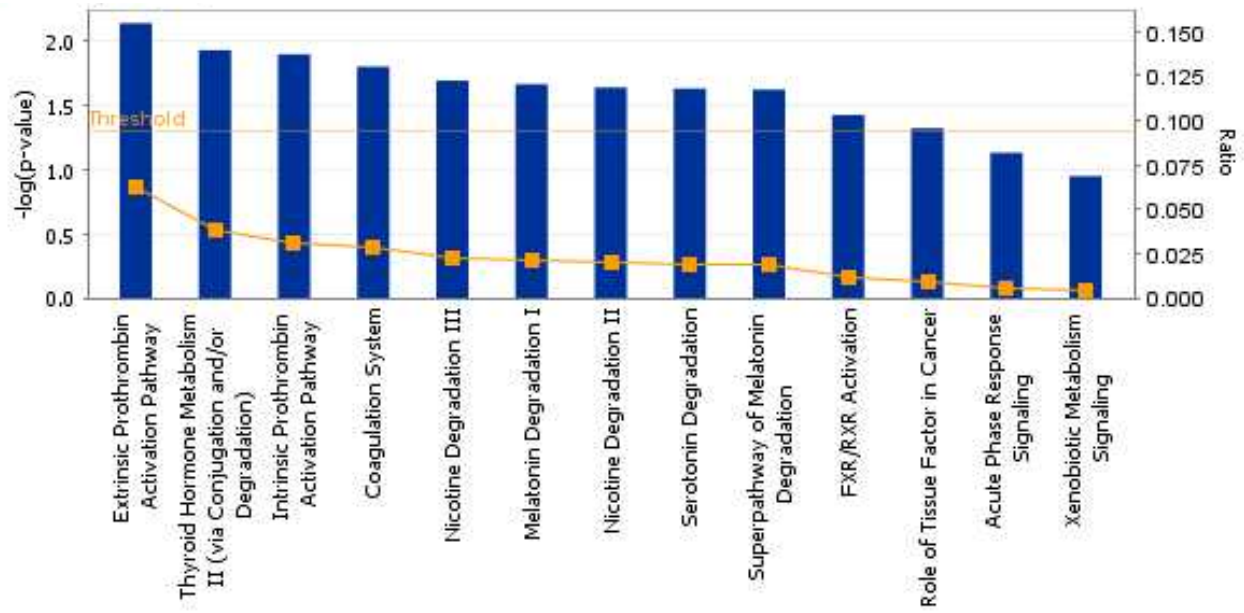
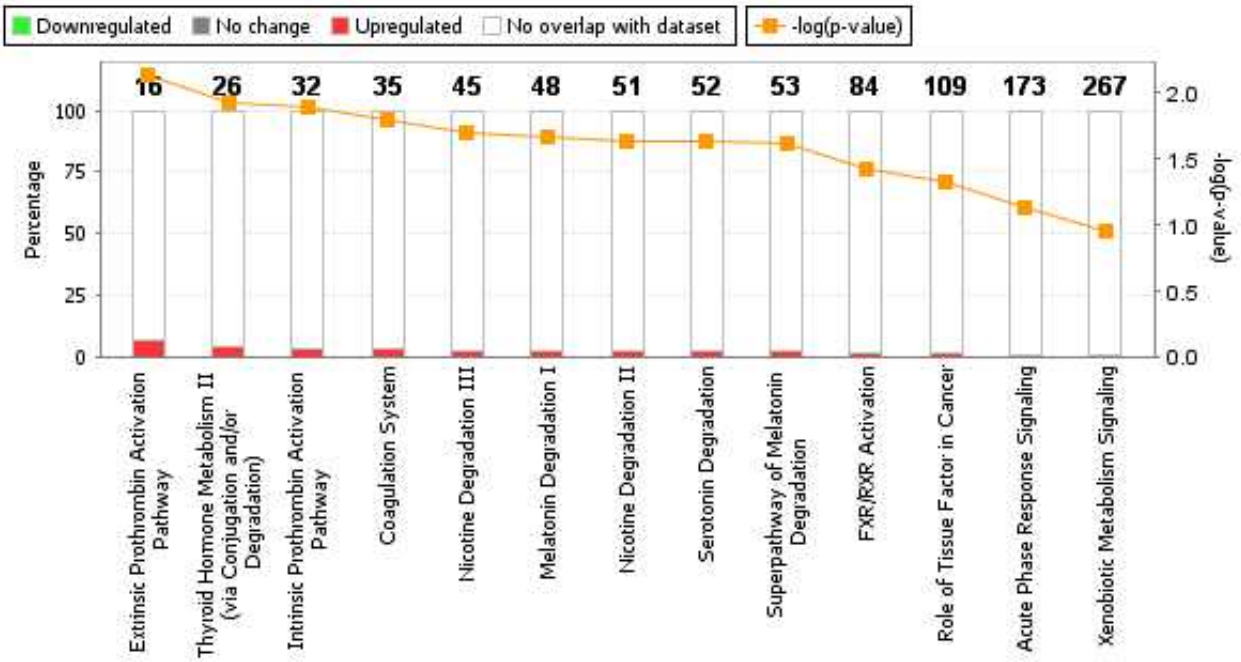


**Figure 7. Percentage of genes expressed in the dataset within the top 13 significantly enriched canonical pathways expressed in normal and tumor tissues**



# CHAPTER V: DISCUSSION

## Potentially harmful effects of soy isoflavones

### *Normal and tumor tissue*

Canonical pathway analysis revealed that genes within the extrinsic and intrinsic prothrombin activation pathways – specifically the fibrinogen alpha chain (FGA) and the fibrinogen beta chain (FGB) genes – were significantly up-regulated by soy isoflavones in tumor tissue. FGA codes for the alpha component of fibrinogen (64); FGB codes for the beta component of fibrinogen (65). One study identified significantly up-regulated coagulation pathways in prostate cancer and benign prostate hyperplasic (BPH) tissue samples (66). The activation of the extrinsic prothrombin pathway was found in the plasma of 106 patients with solid tumors compared with 72 healthy volunteers, and modest intrinsic prothrombin activation was seen only in those cancer patients with advanced disease or in chemotherapy patients (67). Additionally, FGA and FGB were found to be significantly expressed in cancerous tissue of mice with lung cancer (68). Increased FGB expression has been associated with increased tumor stage in bladder cancer (69). These two genes were up-regulated in tumor tissue after exposure to soy isoflavones, suggesting a potentially harmful effect of soy isoflavones in prostate cancer. The FGB gene was also expressed and up-regulated in normal tissue. This pathway and these genes have not been correlated with the effect of soy isoflavones.

***Normal tissue***

Canonical pathway analysis also revealed that the role of IL-17A in psoriasis pathway – specifically the S100 calcium binding protein (S100A7) and chemokine, C-X-C motif (CXCL5) genes – was significantly up-regulated by soy isoflavones in normal prostate tissue. The exact function of the S100A7 molecule has not yet been determined (70); however, over-expression of S100A7 was associated with cell survival in prostate cancer cells derived from tumor tissue, as well as *in vitro* invasion in PC-3 cells (71). The CXCL5 gene codes for chemokines that may promote cancer cell proliferation, migration, and invasion (72). CXCL5 expression was found to correlate with prostate cancer progression (73). The up-regulation of these two genes in normal prostate tissue suggests a potentially harmful effect, though neither gene was present in actual tumor tissue. This pathway and these genes have not been correlated with the effect of soy isoflavones.

**Potentially beneficial effects of soy isoflavones**

***Tumor tissue***

The human embryonic stem cell pluripotency pathway was significantly modulated by soy isoflavones in tumor tissue. The ZIC family member 3 (ZIC3) gene was up-regulated by soy isoflavones in tumor tissue, while the Nanog homeobox (NANOG), the bone morphogenetic protein 5 (BMP5), and the wingless-type MMTV integration site family member 2 (WNT2) genes were down-regulated by soy isoflavones in tumor tissue. None of these genes were expressed in normal prostate tissue **(Table 19)**. The

human embryonic stem cell pluripotency pathway has not been implicated in prostate cancer specifically; however, markers of this pathway have been found to correlate with aggressive tumors (74). Mathieu et al. (74) found a significant correlation between NANOG expression and increased Gleason score in primary prostate tumors. BMP5 and members of the WNT gene family have been implicated in tumor formation (75,76). The ZIC gene family has been found to be significantly expressed in brain tumors (77). Although soy isoflavones up-regulated the ZIC3 gene, suggesting a potentially harmful effect, they also down-regulated the NANOG, BMP5, and WNT2 genes in prostate tumor tissue, suggesting a potentially beneficial effect. This pathway and these genes have not been correlated with the effect of soy isoflavones.

The complement system pathway – specifically the complement factor D (CFD) and the complement component receptor 2 (CR2) genes – were significantly down-regulated by soy isoflavones in tumor tissue. These genes were not expressed in normal tissue **(Table 19)**. CFD codes for a serine protease secreted by adipocytes and belongs to an alternative complement pathway known for its role in humoral suppression of infectious agents (78). One study explored the role of adipose tissue as a source of tumor progenitor cells, and found over-expression of CFD in periprostatic adipose tissue in prostate cancer patients (79). Soy isoflavone supplementation down-regulated this gene in tumor tissue, suggesting a beneficial effect of soy. CR2 codes for an Epstein-Barr virus receptor on B and T lymphocytes (80). The expression of CR2 and other genes related to Epstein-Barr virus infection has been found in tumor tissues of patients with brain cancer, breast cancer, colon cancer, cervical cancer, prostate cancer, and EBV-

negative Hodgkin's lymphoma (81). These two genes have been implicated in cancer development, appeared only in prostate tumor tissue, and were down-regulated by soy isoflavones. This suggests a potentially beneficial effect of soy. This pathway and these genes have not been correlated with the effect of soy isoflavones.

***Normal tissue***

The protein citrullination pathway – specifically peptidyl arginine deiminase, type 3 (PADI3) gene – was significantly down-regulated by soy isoflavones in normal prostate tissue. Protein citrullination involves converting arginine into citrullines (82). Although protein citrullination and the PADI4 gene have been implicated in tumor growth (83,84), the PADI3 gene has been related to skin and hair formation (82). The down-regulation of this gene in prostate cancer tissue samples suggests a potentially beneficial effect of soy isoflavones. This pathway and this gene have not been correlated with the effect of soy isoflavones.

The methylglyoxal degradation VI pathway was highly significant, specifically the lactate dehydrogenase D (LDHD) gene. One study revealed that this pathway was up-regulated in prostate cancer cells, and suggested that it favored cancer cell viability and proliferation (85). Genistein at 12 mg/kg diet was found to inhibit methylglyoxal-induced apoptosis in the mononuclear cells of female Wistar rats (86). The LDHD gene was down-regulated by soy isoflavones in normal prostate tissue, suggesting a potentially beneficial effect of soy isoflavones on prostate cancer progression.

**Unknown role in prostate cancer**

***Tumor tissue***

The acute phase response signaling pathway – specifically the FGB, FGA, lipopolysaccharide binding protein (LBP), and retinol binding protein 4 (RBP4) genes – were significantly modulated by soy isoflavones in tumor tissue. The acute phase response signaling canonical pathway was found to be significantly up-regulated in prostate cancer and benign prostate hyperplasic (BPH) tissue samples (66). As mentioned previously, the FGB gene was significantly up-regulated by soy isoflavones in both normal and tumor tissues. The FGA gene was up-regulated only in tumor tissue, and was not present in normal tissue. The LBP and RBP4 genes were both down-regulated by soy isoflavones in prostate tumor tissue. Neither LBP or RBP4 have been implicated in oncogenesis, but they are both involved in the inflammatory response (87,88). The inflammatory response has been linked to cancer development and progression. Because LBP (89) and RBP4 (90) genes code for pro-inflammatory proteins, the down-regulation of LBP and RBP4 suggest a preventative, potentially beneficial effect of soy isoflavones. Half of the genes expressed in this pathway were related to cancer development and significantly up-regulated, while the other half were related to potential protective effects and were significantly down-regulated. This suggests conflicting effects of soy isoflavones, making it difficult to evaluate the benefit of soy supplementation in prostate cancer. This pathway and these genes have not been correlated with the effect of soy isoflavones.

***Normal tissue***

The glutamate dependent acid resistance pathway was also highly significant. The glutamate acid decarboxylase 2 (GAD2) gene was down-regulated by soy isoflavones in normal prostate tissue. The GAD2 gene, along with the down-regulated gamma-aminobutryic acid receptor 4 (GABRA4) gene, was also present in the GABA receptor signaling pathway. Hudgens et al. found that estradiol stimulated GAD2 expression in rats, the gene that codes for the enzyme primarily responsible for GABA synthesis (91). The structural similarity between soy isoflavones and estrogens may explain how they affected the expression of GAD2 in this study, though one would expect up-regulation instead of down-regulation. The role of these pathways and these genes in prostate cancer development and progression remains unknown. This pathway and these genes have not been correlated with the effect of soy isoflavones.

***Overlapping pathways***

The UDP-glucuronosyltransferase 2 family, polypeptide B4 (UGT2B4) gene was up-regulated by soy isoflavones in both normal and tumor tissues. This gene is a part of the thyroid hormone metabolism III and nicotine degradation pathways, which have not yet been correlated with prostate cancer. Enzymes from UGT2B family affect metabolism and excretion of steroids (92). Though this gene has not been linked with prostate cancer specifically, Lampe et al. (92) found that the prevalence of UGT2B4 was significantly higher in people of Asian descent compared with Caucasians, and that all Asians were homozygous for UGT2B4 compared with Caucasians, potentially explaining

the soy isoflavone intake, Asian population, and reduced prostate cancer incidence link. This pathway and this gene have not been correlated with the effect of soy isoflavones.

**Strengths and limitations**

The majority of this study's limitations originate from the retrospective study design. The quantity and quality of the biospecimen inventory was affected due to lack of control over data storage and collection. Because of this, the prostatectomy samples available for RNA sequencing were limited to 6, rather than the expected 14 prostatectomy samples. As the samples were banked for several years prior to their present use, the storage conditions and therefore the quality of the samples may have been affected. Additionally, the amount of blood lost during surgery and the distribution of epithelial and stromal tissues in the prostatectomy sample have been found to affect the quality of the RNA (18). The parent study did not track these particular variables, but quality control was conducted before sequencing, and all RNA samples had RNA integrity numbers (RIN) over 2.80, indicating good quality.

The choice of isoflavone supplement provided proportionally more daidzein than isoflavone supplements derived from whole soybeans. Whole soy products provide an average isoflavone distribution of 50% genistein, 40% daidzein, and 10% glycitein, while soy germ products provide 15% genistein, 40% daidzein, and 40% glycitein. The majority of reviewed studies focused on products derived from whole soy or on genistein specifically, making it difficult to compare the findings. However, the supplement does

represent a human physiological isoflavone dose so significant effects should be reproducible in humans.

Although the study's sample size was small, the reviewed RNA sequencing human interventions ranged from 3 to 16 participants. A strength of the present study was its use of normal/tumor tissue pairs, as few studies studied the transcriptomes of human tissue and used cell lines or blood cells instead. The relatively short 2 to 4 week dietary intervention period was originally thought to be a limitation, though the majority of interventions employed in the reviewed studies induced a significant change in the transcriptome of cells in as little as 2 weeks. The original protocol did not require an extensive diet history, making it difficult to control for confounding dietary factors.

Human transcriptomic studies are few, especially when focusing specifically on soy isoflavones and prostate cancer. No studies reviewed had investigated the transcriptome of prostate cancer cells using RNA sequencing. Using NGS RNA seq technology provides for much more detailed data.

**Conclusion**

This study provided an opportunity to observe the effects of short-term soy supplementation on the transcriptome in a human model and found that many of the top pathways expressed in normal and cancerous prostate tissue were related to cancer processes and regulated by soy isoflavones. The data provided useful common pathway maps for future understanding of the role of soy isoflavones in prostate cancer prevention, development, and progression.

Soy isoflavones differentially expressed 128 genes in cancerous prostate tissue and 166 genes in normal prostate tissue. Twenty genes were expressed in both cancerous and normal prostate tissue, two of which were differentially regulated by soy isoflavones. Soy isoflavones affected tumor and normal cells differently. Some pathways suggested a protective effect, such as the down-regulation of genes involved in the human embryonic stem cell pluripotency pathway, the complement system pathway, the protein citrullination pathway, and the methylglyoxal degradation VI pathway. Other pathways suggest harm, including the up-regulation of genes involved in the extrinsic and intrinsic prothrombin activation pathways as well as the IL-17A pathway. The following pathways are of unknown relevance: the acute phase response signaling pathway, the UDP-glucuronosyltransferase pathway, and the glutamate dependent acid resistance pathway.

Previous soy isoflavone supplementation interventions in prostate cancer patients have not affected PSA values, hormone profiles, or the incidence of biochemical recurrence, but microarray and RNA sequencing data suggest genetic and molecular changes. The clinical relevance of these findings are still uncertain. Further studies are needed to elucidate the findings and contribute clinically applicable results. Additionally, future studies that compare this study's data with online data repositories would be warranted.

## CHAPTER VI: REFERENCES


1. Siegel R, Naishadham D, Jemal A. Cancer Statistics, 2013. CA Cancer J Clin. 2013;63(1):11–30.
2. Jemal A, Bray F, Ferlay J. Global Cancer Statistics. CA Cancer J Clin. 2011;61(2):69–90.
3. Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology [Internet]. 2010 Aug [cited 2013 May 27];19(8):1893–907. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20647400
4. Messina M, Kucuk O, Lample JW. An overview of the health effects of isoflavones with an emphasis on prostate cancer risk and prostate-specific antigen levels. Journal of AOAC International. 2006;89(4):1121–34.
5. Yuan Y, Ferguson LR. Nutrigenetics and prostate cancer: 2011 and beyond. Journal of nutrigenetics and nutrigenomics [Internet]. 2011 Jan [cited 2013 May 22];4(3):121–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21646812
6. Elliott RM. Transcriptomics and micronutrient research. The British journal of nutrition [Internet]. 2008 Jun [cited 2013 May 5];99 Suppl 3:S59–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18598590
7. Kim J, Yu J. Interrogating genomic and epigenomic data to understand prostate cancer. Biochim Biophys Acta. 2012;1825(2):186–96.
8. Howlander N, Noone A, Krapcho M, Garshell J, Neyman N, Altekruse S, et al. SEER Prostate Cancer Statistics. SEER Cancer Statistics Review, 1975-2010. 2012.

9. Uchio EM, Aslan M, Wells CK, Calderone J, Concato J. Impact of biochemical recurrence in prostate cancer among US veterans. Archives of internal medicine [Internet]. 2010 Aug 9;170(15):1390–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20696967

10. Agarwal PK, Sadetsky N, Konety BR, Resnick MI, Carroll PR. Treatment failure after primary and salvage therapy for prostate cancer: likelihood, patterns of care, and outcomes. Cancer [Internet]. 2008 Jan 15 [cited 2013 May 24];112:307–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18050294

11. American Cancer Society. Prostate cancer [Internet]. 2012. p. 1–85. Available from: http://www.cancer.org/acs/groups/cid/documents/webcontent/003134-pdf.pdf

12. Martens-Uzunova ES, Jalava SE, Dits NF, Van Leenders GJLH, Møller S, Trapman J, et al. Diagnostic and prognostic signatures from the small non-coding RNA transcriptome in prostate cancer. Oncogene [Internet]. 2012 Feb 23 [cited 2013 Feb 28];31(8):978–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21765474

13. Fenech M, El-Sohemy A, Cahill L, Ferguson LR, French T-AC, Tai ES, et al. Nutrigenetics and nutrigenomics: viewpoints on the current status and applications in nutrition research and practice. Journal of nutrigenetics and nutrigenomics [Internet]. 2011 Jan [cited 2013 May 22];4(2):69–89. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3121546&tool=pmcentrez&rendertype=abstract

14. Nature Education. Ribosomes, transcription, and translation. Scitable: Nature. 2013.

15. Kok TMCM De, Breda SGJ Van, Briedé JJ. Genomics-based identification of molecular mechanisms behind the cancer preventive action of phytochemicals: potential and

challenges. Current pharmaceutical biotechnology [Internet]. 2012 Jan;13(1):255–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21466423

16. Perkel JM. Transcriptome analysis: Microarrays, qPCR, and RNA-Seq. Biocompare: The Buyer's Guide for Life Scientists. 2013.

17. Steiner C, Arnould S, Scalbert A, Manach C. Isoflavones and the prevention of breast and prostate cancer: new perspectives opened by nutrigenomics. [Internet]. The British journal of nutrition. 2008 [cited 2013 Mar 1]. p. ES78–108. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18503737

18. Bertilsson H, Angelsen a, Viset T, Anderssen E, Halgunset J. RNA quality in fresh frozen prostate tissue from patients operated with radical prostatectomy. Scandinavian journal of clinical and laboratory investigation [Internet]. 2010 Feb [cited 2013 May 5];70(1):45–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20073672

19. American Cancer Society. Soybean [Internet]. 2013. Available from: http://www.cancer.org/treatment/treatmentsandsideeffects/complementaryandalternativemedicine/dietandnutrition/soybean

20. Barrett JR. The Science of Soy: What Do We Really Know? Environ Health Perspect. 2006;114(6):A352–A358.

21. Soyfoods Association of America. Soy information: Sales and trends. 2011.

22. Messina M, Nagata C, Wu AH. Estimated Asian adult soy protein and isoflavone intakes. Nutrition and cancer [Internet]. 2006 Jan;55(1):1–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16965235

23. U.S. Department of Agriculture. USDA Database for the isoflavone content of selected foods, release 2.0 [Internet]. 2008. Available from: http://www.ars.usda.gov/nutrientdata/isoflav

24. Setchell KDR, Cassidy A. Dietary Isoflavones : Biological Effects and Relevance to Human Health. J Nutr. 1999;129:758S–767S.

25. Nagata C, Inaba S, Kawakami N, Kakizoe T, Shimizu H. Inverse association of soy product intake with serum androgen and estrogen concentrations in Japanese men. Nutrition and cancer [Internet]. 2000 Jan;36(1):14–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10798211

26. Nagata C, Shimizu H, Takami R, Hayashi M, Takeda N, Yasuda K. Association of blood pressure with intake of soy products and other food groups in Japanese men and women. Preventive Medicine [Internet]. 2003 Jun [cited 2013 May 24];36(6):692–7. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0091743503000525

27. Wakai K, Egami I, Kato K, Kawamura T, Tamakoshi A, Lin Y, et al. Dietary intake and sources of isoflavones among Japanese. Nutrition and cancer. 1999;33(2):139–45.

28. Ahmad IU, Forman JD, Sarkar FH, Hillman GG, Heath E, Vaishampayan U, et al. Soy isoflavones in conjunction with radiation therapy in patients with prostate cancer. Nutrition and cancer [Internet]. 2010 Jan [cited 2013 Jul 3];62(7):996–1000. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20924975

29. Gardner CD, Oelrich B, Liu J, Feldman D, Franke AA, Brooks JD. Prostatic soy isoflavone concentrations exceed serum levels after dietary supplementation. Prostate. 2009;69(7):719–26.

30. Swami S, Krishnan A V, Moreno J, Bhattacharyya RS, Gardner C, Brooks JD, et al. Inhibition of prostaglandin synthesis and actions by genistein in human prostate cancer cells and by soy isoflavones in prostate cancer patients. International journal of cancer. Journal international du cancer [Internet]. 2009 May 1 [cited 2013 May 11];124(9):2050–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19127598

31. Kolehmainen M, Mykkänen O, Kirjavainen P V, Leppänen T, Moilanen E, Adriaens M, et al. Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. Molecular nutrition & food research [Internet]. 2012 Oct [cited 2013 Apr 8];56(10):1501–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22961907

32. Ryu M-S, Langkamp-Henken B, Chang S-M, Shankar MN, Cousins RJ. Genomic analysis, cytokine expression, and microRNA profiling reveal biomarkers of human dietary zinc depletion and homeostasis. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2011 Dec 27 [cited 2013 Mar 14];108(52):20970–5. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3248538&tool=pmcentrez&rendertype=abstract

33. Bhamre S, Sahoo D, Tibshirani R, Dill DL, Brooks JD. Temporal changes in gene expression induced by sulforaphane in human prostate cancer cells. Prostate. 2009;69(2):181–90.

34. Takahashi Y, Konishi T. Tofu (soybean curd) lowers serum lipid levels and modulates hepatic gene expression involved in lipogenesis primarily through its protein, not isoflavone, component in rats. Journal of agricultural and food chemistry [Internet]. 2011

Aug 24;59(16):8976–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21721588

35. Siler U, Barella L, Spitzer V, Schnorr J, Lein M, Goralczyk R, et al. Lycopene and vitamin E interfere with autocrine/paracrine loops in the Dunning prostate cancer model. FASEB journal : official publication of the Federation of American Societies for Experimental Biology [Internet]. 2004 Jun [cited 2013 May 5];18(9):1019–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15084515

36. Iqbal MJ, Yaegashi S, Ahsan R, Lightfoot D a, Banz WJ. Differentially abundant mRNAs in rat liver in response to diets containing soy protein isolate. Physiological genomics [Internet]. 2002 Dec 3 [cited 2013 Mar 4];11(3):219–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12388795

37. Takahashi Y, Ide T. Effects of soy protein and isoflavone on hepatic fatty acid synthesis and oxidation and mRNA expression of uncoupling proteins and peroxisome proliferator-activated receptor gamma in adipose tissues of rats. The Journal of nutritional biochemistry [Internet]. 2008 Oct [cited 2013 May 7];19(10):682–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18328687

38. Boomgaarden I, Egert S, Rimbach G, Wolffram S, Müller MJ, Döring F. Quercetin supplementation and its effect on human monocyte gene expression profiles in vivo. The British journal of nutrition [Internet]. 2010 Aug [cited 2013 Mar 26];104(3):336–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20416132

39. Pagmantidis V, Méplan C, Van Schothorst EM, Keijer J, Hesketh JE. Supplementation of healthy volunteers with nutritionally relevant amounts of selenium increases the

expression of lymphocyte protein biosynthesis genes. The American journal of clinical nutrition [Internet]. 2008 Jan;87(1):181–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18175754

40. Niculescu MD, Pop E a, Fischer LM, Zeisel SH. Dietary isoflavones differentially induce gene expression changes in lymphocytes from postmenopausal women who form equol as compared with those who do not. The Journal of nutritional biochemistry [Internet]. 2007 Jun [cited 2013 Mar 1];18(6):380–90. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2441946&tool=pmcentrez&rendertype=abstract

41. Xu L, Ding Y, Catalona WJ, Yang XJ, Anderson WF, Jovanovic B, et al. MEK4 function, genistein treatment, and invasion of human prostate cancer cells. Journal of the National Cancer Institute [Internet]. 2009 Aug 19 [cited 2013 May 11];101(16):1141–55. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2728746&tool=pmcentrez&rendertype=abstract

42. Traka M, Gasper A V, Smith JA, Hawkey CJ, Bao Y, Mithen RF. Nutrient-Gene Interactions Transcriptome Analysis of Human Colon Caco-2 Cells Exposed. Am J Clin Nutr. 2005;135(8):1865–72.

43. Thangapazham RL, Shaheduzzaman S, Kim K-H, Passi N, Tadese A, Vahey M, et al. Androgen responsive and refractory prostate cancer cells exhibit distinct curcumin regulated transcriptome. Cancer biology & therapy [Internet]. 2008 Sep;7(9):1427–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18719366

44. Pitchakarn P, Suzuki S, Ogawa K, Pompimon W, Takahashi S, Asamoto M, et al. Kuguacin J, a triterpeniod from Momordica charantia leaf, modulates the progression of androgen-independent human prostate cancer cell line, PC3. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association [Internet]. Elsevier Ltd; 2012 Mar [cited 2013 May 5];50(3-4):840–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22266361

45. Kelly L a, O'Leary JJ, Seidlova-Wuttke D, Wuttke W, Norris L a. Genistein alters coagulation gene expression in ovariectomised rats treated with phytoestrogens. Thrombosis and haemostasis [Internet]. 2010 Dec [cited 2013 May 7];104(6):1250–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20838740

46. Li Y, Sarkar FH. Gene expression profiles of genistein-treated PC3 prostate cancer cells. The Journal of Nutrition [Internet]. 2002 Dec;132(12):3623–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12468598

47. Suzuki K, Koike H, Matsui H, Ono Y, Hasumi M, Nakazato H, et al. Genistein, a soy isoflavone, induces glutathione peroxidase in the human prostate cancer cell lines LNCaP and PC-3. International journal of cancer. Journal international du cancer [Internet]. 2002 Jun 20 [cited 2013 May 7];99(6):846–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12115487

48. Skogseth H, Follestad T, Larsson E, Halgunset J. Transcription levels of invasion-related genes in prostate cancer cells are modified by inhibitors of tyrosine kinase. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica [Internet]. 2006 May;114(5):364–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16725013

49. Majid S, Dar A a, Shahryari V, Hirata H, Ahmad A, Saini S, et al. Genistein reverses hypermethylation and induces active histone modifications in tumor suppressor gene B-Cell translocation gene 3 in prostate cancer. Cancer [Internet]. 2010 Jan 1 [cited 2013 Apr 17];116(1):66–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19885928

50. Xiao R, Badger TM, Simmen F a. Dietary exposure to soy or whey proteins alters colonic global gene expression profiles during rat colon tumorigenesis. Molecular cancer [Internet]. 2005 Jan 11 [cited 2013 Apr 29];4(1):1. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=545049&tool=pmcentrez&rendertype=abstract

51. Zhang J, Lazarenko OP, Wu X, Tong Y, Blackburn ML, Gomez-Acevedo H, et al. Differential effects of short term feeding of a soy protein isolate diet and estrogen treatment on bone in the pre-pubertal rat. PloS one [Internet]. 2012 Jan [cited 2013 May 7];7(4):e35736. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3335011&tool=pmcentrez&rendertype=abstract

52. Franke AA, Custer LJ, Wilkens LR, Le Marchand L Le, Nomura AMY, Goodman MT, et al. Liquid chromatographic-photodiode array mass spectrometric analysis of dietary phytoestrogens from human urine and blood. Journal of chromatography B [Internet]. 2002 Sep 25;777(1-2):45–59. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12270199

53. Setchell KDR, Cole SJ. Method of defining equol-producer status and its frequency among vegetarians. The Journal of nutrition [Internet]. 2006 Aug;136(8):2188–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16857839

54. University of Kansas Medical Center. Protocol for RNA isolation using TRIzol Reagent with Phase Lock GelHeavy [Internet]. Kansas City, KS; Available from: http://www2.kumc.edu/siddrc/microarray/PhaseLockGelprotocol.pdf

55. University of Kansas Medical Center Genome Sequencing Facility. Illumina TruSeq RNA Sample Prep (LT) Protocol [Internet]. Kansas City, KS; Available from: http://www.kumc.edu/Documents/gsf/illumina TruSeq RNA Protocol.pdf

56. Babraham Bioinformatics. FastQC [Internet]. Cambridge, UK: Babraham Institute; 2012. Available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

57. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome biology [Internet]. BioMed Central Ltd; 2013 Apr 25 [cited 2013 May 21];14(4):R36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23618408

58. Aronesty E. Command-line tools for processing biological sequencing data [Internet]. 2011. Available from: http://code.google.com/p/ea-utils

59. DeLuca DS, Levin JZ, Sivachenko A, Fennell T, Nazaire M-D, Williams C, et al. RNA-SeQC: RNA-seq metrics for quality control and process optimization. Bioinformatics (Oxford, England) [Internet]. 2012 Jun 1 [cited 2013 May 22];28(11):1530–2. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3356847&tool=pmcentrez&rendertype=abstract

60. Anders S. HTSeq. 2010.

61. Ensembl. Human assembly and gene annotation. Ensembl release 72. 2013.

62. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2013. Available from: http://www.r-project.org/

63. Hamilton-Reeves JM, Banerjee S, Banerjee S, Holzbeierlein J, Thrasher JB, Kambhampati S, et al. Short-term soy isoflavone intervention in patients with localized prostate cancer: a randomized, double-blind, placebo-controlled trial. PloS one.

64. FGA fibrinogen alpha chain. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013.

65. FGB fibrinogen beta chain. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013.

66. Savli H, Szendröi A, Romics I, Nagy B. Gene network and canonical pathway analysis in prostate cancer: a microarray study. Experimental & molecular medicine [Internet]. 2008 Apr 30;40(2):176–85. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2679302&tool=pmcentrez&rendertype=abstract

67. Kakkar A, DeRuvo N, Chinswangwatanakul V, Tebbutt S, Williamson R. Extrinsic-pathway activation in cancer with high factor VIIa and tissue factor. The Lancet [Internet]. 1995;346(8981):1004–5. Available from: http://search.proquest.com/docview/198968962?accountid=28920

68. Choi J-W, Liu H, Song H, Park JHY, Yun JW. Plasma marker proteins associated with the progression of lung cancer in obese mice fed a high-fat diet. Proteomics [Internet]. 2012 Jun [cited 2013 Jun 27];12(12):1999–2013. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22744879

69. Lindén M, Segersten U, Runeson M, Wester K, Busch C, Pettersson U, et al. Tumour expression of bladder cancer-associated urinary proteins. BJU international [Internet]. 2013 Mar 7 [cited 2013 Jun 27];1–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23470167

70. S100A7 S100 calcium binding protein A7. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013.

71. Ye L, Sun P-H, Martin T a, Sanders AJ, Mason MD, Jiang WG. Psoriasin (S100A7) is a positive regulator of survival and invasion of prostate cancer cells. Urologic oncology [Internet]. 2012 Jun 11 [cited 2013 Jun 27];1–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22694938

72. CXCL5 chemokine (C-X-C motif) ligand 5. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013.

73. Begley LA, Kasina S, Mehra R, Adsule S, Admon AJ, Lonigro RJ, et al. CXCL5 Promotes Prostate Cancer Progression. Neoplasia. 2008;10(3):244–54.

74. Mathieu J, Zhang Z, Zhou W, Wang AJ, Heddleston JM, Pinna CMA, et al. HIF induces human embryonic stem cell markers in cancer cells. Cancer Res. 2011;71(13):4640–52.

75. BMP5 bone morphogenetic protein 5. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013.

76. WNT2 wingless-type MMTV integration site family member 2. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013.

77. Aruga J, Nozaki Y, Hatayama M, Odaka YS, Yokota N. Expression of ZIC family genes in meningiomas and other brain tumors. BMC cancer [Internet]. 2010 Jan;10:79. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2838823&tool=pmcentrez&rendertype=abstract

78. CFD complement factor D (adipsin) [Internet]. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013. Available from: http://www.ncbi.nlm.nih.gov.proxy.kumc.edu:2048/gene/1675

79. Ribeiro R, Monteiro C, Silvestre R, Castela A, Coutinho H, Fraga A, et al. Human periprostatic white adipose tissue is rich in stromal progenitor cells and a potential source of prostate tumor stroma. Experimental biology and medicine (Maywood, N.J.) [Internet]. 2012 Oct 1 [cited 2013 Jun 27];237(10):1155–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23038706

80. CR2 complement component (3d/Epstein Barr virus) receptor 2. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013.

81. Michaelis M, Doerr HW. The Story of Human Cytomegalovirus and Cancer: Increasing Evidence and Open Questions. Neoplasia. 2009;11(1):1–9.

82. PADI3 peptidyl arginine deiminase, type III [Internet]. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013. Available from: http://www.ncbi.nlm.nih.gov.proxy.kumc.edu:2048/gene/51702

83. Chang X, Han J, Pang L, Zhao Y, Yang Y, Shen Z. Increased PADI4 expression in blood and tissues of patients with malignant tumors. BMC cancer [Internet]. 2009 Jan [cited 2013 Jun 27];9:40. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2637889&tool=pmcentrez&rendertype=abstract

84. Chang X, Fang K. PADI4 and tumourigenesis. Cancer cell international [Internet]. 2010 Jan;10:7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2845578&tool=pmcentrez&rendertype=abstract

85. De Bari L, Moro L, Passarella S. Prostate cancer cells metabolize d-lactate inside mitochondria via a D-lactate dehydrogenase which is more active and highly expressed than in normal cells. FEBS letters [Internet]. 2013 Mar 1 [cited 2013 May 22];587(5):467–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23333299

86. Wu H-J, Chan W-H. Genistein protects methylglyoxal-induced oxidative DNA damage and cell injury in human mononuclear cells. Toxicology in vitro : an international journal published in association with BIBRA [Internet]. 2007 Apr [cited 2013 Jul 3];21(3):335–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17049802

87. RBP4 retinol binding protein 4. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013.

88. LBP lipopolysaccharide binding protein. Bethesda, MD: National Center for Biotechnology, US National Library of Medicine; 2013.

89. Heumann D, Bas S, Gallay P, Le Roy D, Barras C, Mensi N, et al. Lipopolysaccharide binding protein as a marker of inflammation in synovial fluid of patients with arthritis: correlation with interleukin-6 and C-reactive protein. J Rheumatol. 1995;22(7):1224–9.

90. Farjo KM, Farjo R a, Halsey S, Moiseyev G, Ma J-X. Retinol-binding protein 4 induces inflammation in human endothelial cells by an NADPH oxidase- and nuclear factor kappa B-dependent and retinol-independent mechanism. Molecular and cellular biology [Internet]. 2012 Dec [cited 2013 Jul 3];32(24):5103–15. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3510526&tool=pmcentrez&rendertype=abstract

91. Hudgens ED, Ji L, Carpenter CD, Petersen SL. The gad2 promoter is a transcriptional target of estrogen receptor a (ERa) and ERB: A unifying hypothesis to explain diverse effects of estradiol. J Neurosci. 2009;29(27):8790–7.

92. Lampe JW, Bigler J, Bush AC, Potter JD. Prevalence of polymorphisms in the human UDP-glucuronosyltransferase 2B family: UGT2B4(D458E), UGT2B7(H268Y), and UGT2B15(D85Y). Cancer Epidemiol Biomarkers Prev. 2000;9:329–33.